Synthesis strategies to 3-alkyl-2-methoxy pyrazines

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By

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Declaration

The work in this thesis is based on research carried out within the Baxendale laboratory, in the Department of Chemistry at Durham University, UK. No part of this thesis has been submitted elsewhere for any other degree or qualification and it is the author's own work unless indicated otherwise in the text; for example, where the referential work of previous project is taken as a reference.

Acknowledgements

Thanks to all the experience gained during the master's education which has equipped me with more comprehensive research skills. I have gained more passion for organic synthesis, as always. Thanks to all the books which have supported me to practice writing skills and logical thinking. Thanks to all the dairies which have given me the record to recognise daily improvements and confidence in continuing my research career. A big thank you to all the people who supported and accompanied me with courage and wisdom. Thanks to my idol Yi Yangqianxi, who makes me believe that we can always chase our dream with endless trust in ourselves. Thanks to myself for being resilient, and please keep going.

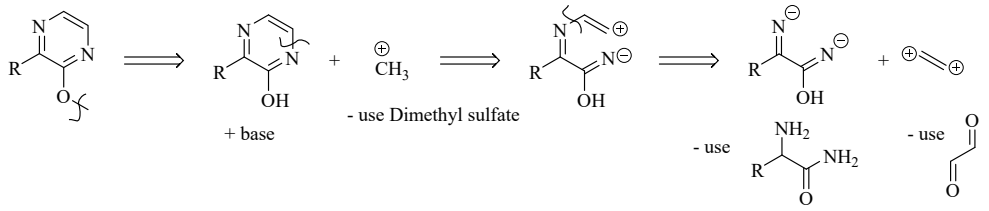
Abstract

Pyrazines are a class of heterocyclic compounds with multiple industrial applications spanning the flavours, fragrance, and pharmaceutical industries. The investigation of new synthetic routes leading to improved safety, efficiency and cost are advantageous to large scale synthesis in each of these sectors. One of our laboratories principle ambitions is to establish generic methodology that allows access to a wide range of novel heterocyclic cores such as pyrazines. To develop this chemistry, we selected three exemplifying structures namely, 2-butyl-3-methoxypyrazine, 2-propyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine, with the eventual aim of enabling large scale production.

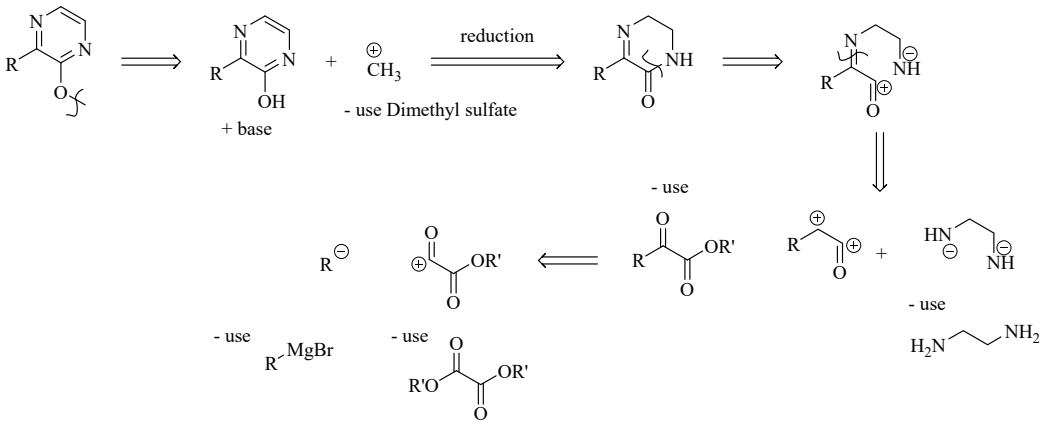
Prior to describing the synthesis of these compounds, a background literature review of current pyrazine preparation is provided. This includes the unique odour characters of this family, their application in the flavour and fragrance area and biological impacts as semiochemicals. A more comprehensive pyrazine natural formation also provided some insights and data to estimate the reaction conditions and starting materials required for the new synthesis.

The retrosynthesis of 2-alkoxy-3-methoxypyrazines is shown in the abstract figure outlined below. Firstly, the determination of key variables relating to yield based upon a known synthetic route suggested by Seifert *et al*. in 1970, was investigated.[1] Further literature sources were also reviewed to identify improvements for each step of the originally established scheme. Overall, around 50% yield of the selected target 2-isobutyl-3-methoxypyraizne were obtained and under milder reaction conditions. Secondly, a new route was considered for the synthesis of 2-isobutyl-3-methxoypyrazine via condensation of α,β-dicarbonyl and of α,β-diamine compounds. The method was based upon an approach by Ghosh *et al*. published in 2011.[2] The scope of this route was analysed and was adjusted to enable us to prepare 2-isobutyl-3-methoxypyrazines via a novel aromatisation procedure.

Route 1



Route 2



Abbreviations

δ	chemical shift (NMR spectroscopy)
v	wavenumber (IR spectroscopy)
br.	broad (NMR spectroscopy)
cm^{-1}	inverse centimetre
d	doublet (NMR spectroscopy)
DEPT	distortionless enhancement through polarisation transfer
DMSO	dimethylsulfoxide
d.r.	diastereomeric ratio
equiv.	equivalents
et al.	*et alia*
FD	Flavour Dilution
FT-IR	Fourier-transform infrared
g	grams
GCO	gas chromatography olfactometry
h	hours
HRMS	high resolution mass spectrometry
Hz	Hertz
J	coupling constant (NMR spectroscopy)
LC-MS	liquid chromatography mass spectrometry
m	medium intensity (IR spectroscopy)
m	multiplet (NMR spectroscopy)
M	moles per litre
min	minutes

mL	millilitres
mol	moles
nm	nanometres
NMR	nuclear magnetic resonance
OT	Odour threshold
OTV	Odour threshold value
ppm	parts per million
q	quartet (NMR spectroscopy)
R_t	retention time
r.t.	room temperature
s	strong intensity (IR spectroscopy)
t	triplet (NMR spectroscopy)
THF	tetrahydrofuran
tlc	thin layer chromatography
w	weak intensity (IR spectroscopy)

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Figure 15. The proposed biosynthetic pathway for 2-ethyl-3,5(3,6)-dimethylpyrazine in Bacillus subtilis. TDH: *L*-theronine-3-dehydrogenase; TDA: *L*-threonine deaminase; AMDH: amine dehydrogenase.

Figure 16. Two proposed biosynthetic pathways for 2-alkyl-3-methoxypyrazine (21). OMT: O-methyltransferase. (a) Biosynthesis with leucine and glyoxal proposed by Murray *et al*. 1970; (b) Biosynthesis with valine and glycine proposed by Cheng *et al*. 1991. No biochemical evidence has confirmed the pathway with dashed arrows.

Figure 17. The set-up of the procedure to synthesis L-leucinamide hydrochloride. The condenser is stoppered with a septum and a nitrogen balloon on the top to form a closed system with controlled building pressure.

Figure 18. The crystal structure of the colourless structure and was confirmed as leucine crystals.

Figure 19. The structure of *L*-isoleucine methyl ester hydrochloride and *L*-isoleucine ethyl ester hydrochloride.

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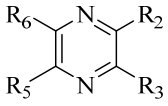
1. Introduction of Pyrazines

Pyrazines are six membered heterocyclic compounds containing two *para* (1,4) positioned ring nitrogen atoms with functional groups attached to carbons on the other aromatic ring positions. These are represented by R_2 R_3 R_5 and R_6 in Table 1, where each represents a different functional group such as hydrogen atom, alkyl, alkoxy, aryl group etc. The substituents have been grouped in four categories, from no carbon chains to carbon chains and carbon chains with heteroatoms.[3] Exemplifying the metabolic detoxification pathways in pyrazine synthesis via oxidation of side-chain alkyl or oxygenated functional groups and hydroxylation of the ring (Figure 1).[3] Alkylpyrazines are also more prevalent in heat-induced process while methoxypyrazines from natural fermentation process as illustrated later in Section 1.3.

Table 2. The classification of pyrazine derivatives suggested by Adams *et al*. 2002.[3]



Entry	Categories	Substituted group
1	Unsubstituted pyrazine	R = H
2	Pyrazine derivatives possessing hydrocarbon substituent	R = alkyl, alicyclic, aryl, etc.
3	Oxygenated pyrazines	R = OR, CHO, etc.
4	Pyrazine derivatives having a thiol or thioether functional group	R = RSH or SR_2

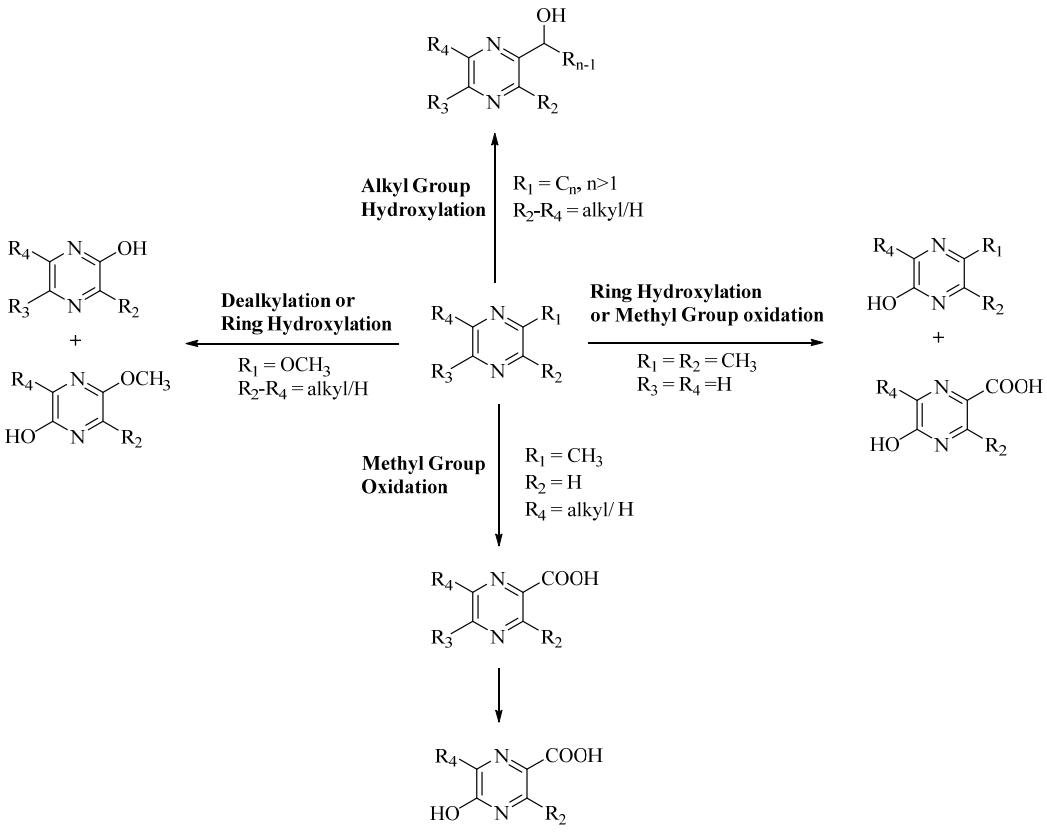
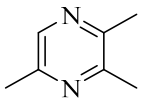
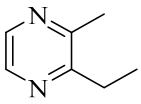
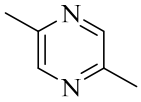
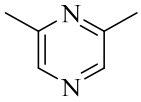
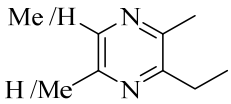
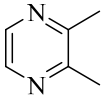
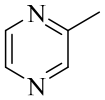


Figure 1. The metabolic detoxication pathways of alkyl- and alkoxy- substituted pyrazine derivatives. Adapted from Adams *et al*. 2001.[3]

This heterocyclic class of pyrazines possess a plethora of application being present in many foods, fragrances, and pharmaceuticals.[4, 5, 6, 7] Alkylated pyrazines are generated extensively in food processing by heat induced process such as the Maillard Reaction.[8] A variety of these products are odorous and can be detected as fruity, chocolate-like, and nutty odours. The Generally Recognized as Safe (GRAS) ingredients are often used to enhance some food flavours in industry.[3] Methoxypyrazines have been found as semiochemicals in prey and predator relationships. These are also found to be generated in several fermented food products such as wines, liquors and teas under mild conditions.[9] Specifically, 2-methoxy-3-isobutylpyrazine is a key ingredient of the petitgrain base in perfume.[10] Furthermore, pyrazinamide and their derivatives are well-known as antimycobacterial agents, and have found use in the treatment of tuberculosis and as antiallergic agents.[7] The global market demand for pyrazine derivatives has only increased the requirement for even more efficient preparation since the first publication of the recorded pyrazine synthesis – tetraphenylpyrazine by Laurent in

1855.[11] The annual usage of the most processed alkyl pyrazine as flavouring agents in Europe in 2004 are shown in Table 2. In 2019, the global flavour and fragrance market had reached over $25.5 billion.[6]

Table 2. The annual usage of the most processed alkyl pyrazines as flavouring agents in Europe in 2004.[6]

Entry	Pyrazine	Annual Usage/ kg
1		840
2		590
3		156
4		11
5		309
6		112
7		139

In the following introduction to pyrazines our general aim has been to indicate the importance of this class of heterocycle. In addition, we note that some further literature is discussed when considering the syntheses of these materials which is provided in section 2, pertaining to the Results and Discussion section. For those wishing more in-depth study we recommend the following comprehensive reviews of pyrazine compound.[7, 11-12]

1.1 Odour characters of pyrazines

1.1.1 Adjective description of odourants

The sensory characteristics of pyrazine derivatives are well-known in the food

flavourings and fragrance industries. These derivatives present a wide range of descriptors as adjectives summarised from daily impressions. For instance, a Scotch whisky flavour was reviewed by Lee *et al*. 2000 and summarised into 14 categories of pyrazine derived tastes including, peaty, grainy, grassy, fruity, floral, reints, woody, sweet, stale, sulfury, cheesy, oily, regarding primary taste and mouth nasal effect.[13] This form of description is also widely found in commercial products such as coffee,[14] wine and perfume[15] to provide relatively more clarified descriptions for customers. Other studies have investigated the influence of chirality on odour descriptors (Table 3).[8] By changing the chirality of a compound, its odour descriptor can switch between daily impression of "non-related" known products. The commonly encountered exemplification of this is the different enantiomers of Carvone which present a nose of either caraway or spearmint.

Table 3. The odour descriptors of some selected chiral compounds.[8]

Compounds	**Odour descriptor**
Linalool	(+) Sweet, petigrain
	(-) Woody, lavender
Carvone	(+)- Caraway
	(-) Spearmint
Nootkatone	(+) Grapefruit
	(-) Woody, spicy
Nerol oxide	(+) Green, floral
	(-) Green, spicy, geranium
Menthol	(+) Dusty, vegetable, less minty, less cooling than (-)
	(-) Sweet, fresh minty, strong cooling effect
Limonene	(+) Orange
	(-) Turpentine

1.1.2 Qualitative analysis of odourants

Quantitative analyses methods have also been developed for odour description to detect and/ or recover a particular combinatorial code of flavour/sent.[16] For instance, in Food Flavours, chemical, sensory and technological properties can be captured using *Detection Threshold* and *Odour Threshold* are identified and stated as [8]:

- Detection threshold: "The lowest physical intensity at which a stimulus is perceptible."
- Odour threshold (or recognition threshold): "The lowest intensity in which the stimulus can be correctly defined or identified."

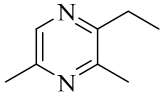
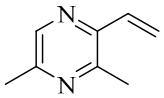
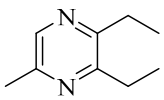
The latter *Odour Threshold* term[17] is frequently used to quantify the contribution of aroma compounds and it can be measured in air (T_a), water (T_w) or oil (T_{ol}) as shown in Equation 1 and Equation 2. Where K_{aw} is the air-to-water partition of the compound at the testing temperature, and K_{aol} is the oil-to-water partition.

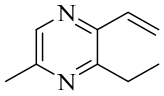
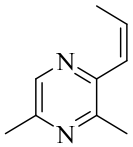
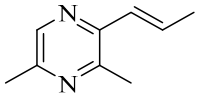
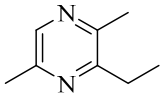
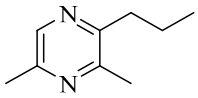
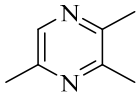
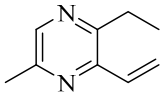
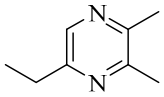
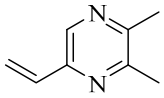
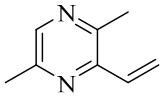
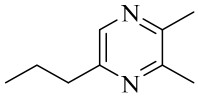
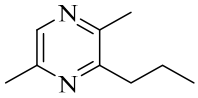
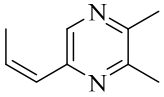
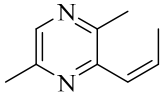
$$T_a = T_w \times K_{aw} \qquad \textbf{Equation 1.}$$

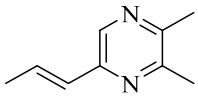
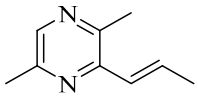
$$T_{ol} = T_w \times \left(\frac{K_{aw}}{K_{aol}}\right) \qquad \textbf{Equation 2.}$$

In 2001, Adams *et al*. determined the Odour quality (OQ) and Odour threshold (OT) of over 80 different alkyl pyrazines which illustrated an extensive range of Odour threshold values from 0.011 to > 2000 ng L^{-1} in air (Table 4).[3]

Table 4. Examples of odour description and Odour threshold values (OTVs) of some alkyl pyrazines.[3]

Pyrazine	No.	Odour description	Odour threshold (ng/l in air)
	**1**	Roasty, earthy	0.011
	**2**	Earthy	0.012
	**3**	Earthy	0.014

	**4**	Earthy	0.014
	**5**	Earthy	0.057
	**6**	Earthy	1.8
	**7**	Roasty, earthy	3.6
	**8**	Earthy	24
	**9**	Roasty	50
	**10**	Earthy	110
	**11**	Roasty, earthy	200
	**12**	-	> 500
	**13**	Earthy	870
	**14**	-	> 1200
	**15**	-	> 2000
	**16**	-	> 2000
	**17**	-	> 2000

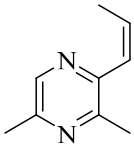
	**18**	-	> 2000
	**19**	-	> 2000

Besides odour detection in various food and flavouring ingredients, the taste of the food can also be enhanced or reconstructed through aroma extraction dilution analysis (AEDA).[17]

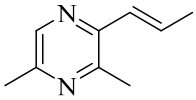
The quantitative ratio of active aroma compounds is achieved by screening concentration decrease in active aroma compounds with continuously diluted samples of a defined set of aromas.[18-19] In other words, the most potent compounds will be screened off first and the rest in stepwise dilution procedures. The diluted extracts are evaluated by GCO and recorded as Flavour Dilution (FD) factor (i.e., the maximum dilution of an extract at which the compound can be detected). The method has been applied to evaluate wheat and rye bread crusts, Doubanjiang (a Chinese red pepper paste), filter and instant coffee, and etc.[19-21]

1.1.3 Perception of odorants

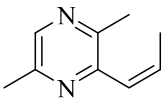
In addition to the concentration of the odourants, the perception of odorants by olfactory system is another curial factor in the sensory characterisation of smell.[8] The effect of stereoisomers on Odour Threshold values can be seen by comparing compounds **5**, **6**, **17** and **19** (Figure 2). All four pyrazines have disubstituted methyl group and a propenyl group attached where **17**, **19** and **5**, **6** are stereoisomers respectively. Pyrazines **17** and **19** both gave OT > 2000 ng/l in air, **5** and **6** both possess an earthy odour with an approximately 32-fold difference in odour intensity.[16]



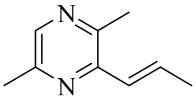
3,5-Dimethyl-2-(*Z*-1-propenyl)pyrazine (**5**)
OT = 0.057 ng/l



3,5-Dimethyl-2-(*E*-1-propenyl)pyrazine (**6**)
OT = 1.8 ng/l in air



2,5-Dimethyl-3-(*Z*-1-propenyl)pyrazine (**17**)
OT > 2000 ng/l

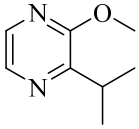
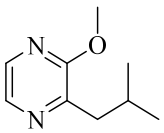
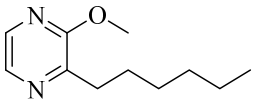


2,5-Dimethyl-3-(*E*-1-propenyl)pyrazine (**19**)
OT > 2000 ng/l

Figure 2. The odour thresholds (OT) of chiral compounds **5** and **6**, **17** and **19**.[16]

In addition to alkyl pyrazines, Seifert *et al.* in 1970[1] analysed a collection of 3-alkyl-2-methoxypyrazines (Table 5), a special class of fragrance components with unique bell pepper odour and very low Order Thresholds (compare to all other alkyl pyrazines in this report). The Odour Potency of 2-isopropyl-3-methoxyoyrazine (**20**), 2-isobutyl-3-methoxypyrazine (**21**) and 2-*sec*-butyl-3-methoxypyrazine (**22**) was also verified by Murray *et al*. 1970[22] who isolated these methoxypyrazines from green peas.

Table 5. The odour thresholds of three methoxypyrazines with bell-pepper odour.[1]

Pyrazine	**No.**	**Odour threshold in Parts of Compound per 10^{12} Parts of Water**	**The extend of similarity to bell pepper odour**
	**20**	6	Moderate
	**21**	2	Very
	**64**	1	very

Because of their wealth of properties, the stereoisomerism, position of heteroatoms, and overall composition of substituent groups have been widely investigated and compared to develop a structure-odour relationships for compounds with bell-pepper notes. An excellent representative investigation along these lines has been published by Rognon and Chastrette in 1994 (Figure 3).[23]

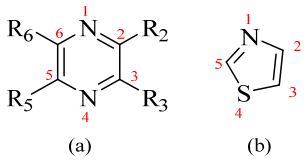


Figure 3. General structure of (a) Pyrazines and (b) Thiazoles in structure-odour relationship study by Rognon and Chastrette in 1994.[23]

The tri-dimensional prediction model developed was assembled from analysis of the low-energy conformation of molecules, interaction between molecules and active sites, and their corresponding odour intensity detection. The hydrogen bonds and dispersion forces were the main interactions involved between these molecules as stated in hydrogen bonding and dispersion theory (HBD); the minimal conformation of molecules was computed by molecular mechanics software (MMP2); the olfactory threshold was recorded in ppm in water.

Overall, the molecular site interaction had a direct relationship with the number of molecular characteristics, consequently an inverse effect with olfactory thresholds. The olfactory thresholds were influenced by qualitative molecular interactions. The suggested optimum substituent at the 2-position for generating the bell-pepper odour was identified as a branched alkyl group of between three to eight carbon atoms, a group of one to three carbon atoms or heteroatoms is preferred at 3-position and no substituents at the 5 and 6 position which increases the bell-pepper intensity, especially when the carbon atom at the 6-position is unhindered. The structural fragment model involving one sp^2 nitrogen and sp^2 carbons is shown in Figure 4. This fragment pattern was suggested with better superimposition and consistency with olfactory results obtained during analysis by Rognon and Chastrette 1994.[23]

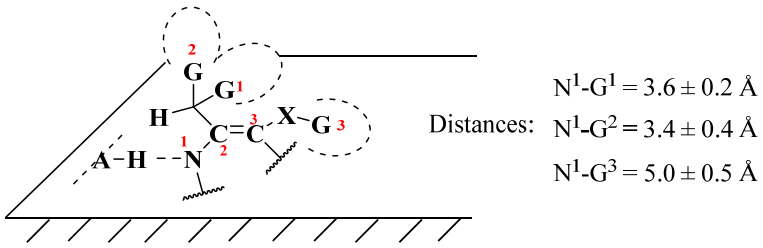


Figure 4. The tri-dimensional structural fragment model of interactions between odoriferous and olfactory receptor sites of the bell-pepper notes by Rognon and Chastrette 1994. This included one nitrogen and two carbon atoms, all of sp^2 hybridisation.[23]

The structural contribution of heteroatoms on attached substituents was also noted. While 2-alkoxypyrazines such as 2-isobutyl-3-methoxypyrazine were found with relatively intense bell-pepper odour, the alkoxy groups were not specifically involved in direct hydrogen bonding interactions. Further evidence showed the replacement of the alkoxy group with an alkyl group at the position 2, such as 2-isobutyl-3-methylpyrazine gave a change in olfactory threshold from 3.5×10^{-2} [24] to 1.3×10^{-1} [25] ppm.

The intense odour property of methoxypyrazines were investigated by comparing conformational variation with energy difference of the most stable conformation of 2-isobutyl-3-methoxypyrazine (**21**) (Figure 5). The dihedral angle of the methoxy group was shown to be 180° at conformations of two steric energy minima. This structure and odour relationship could be explained by the induced conjugation of lone pairs on the oxygen into the aromatic ring which constrains the methoxy group in the plane creating a specific structural pattern.

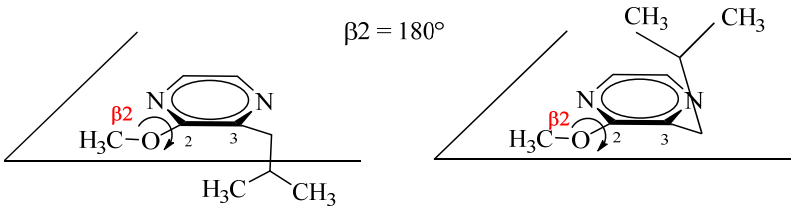


Figure 5. Two conformations at the steric energy minima of 2-isobutyl-3-methoxypyrazine (**21**). B2 is the dihedral angle recorded for rotation of methoxy group.

1.2 Flavour, Fragrance, and biological applications of pyrazines

Substituted pyrazines such as alkyl pyrazines and methoxypyrazines are widely distributed in nature. A number of study have shown their occurrence in vegetables,[3]

insects,[26, 27, 28] host plants of insects,[28] mammals[29, 30] and amphibians.[31]

1.2.1 Pyrazine compounds in foods, wines, and perfumes

In foods

Some pyrazines have stimulating and desirable food flavour characteristics and have been isolated from raw or roasted foods such as tomatoes, hydrolysed soy protein, potato chips, nuts, and coffee to list only a few (Table 6). Extracted or synthesised pyrazine derivatives can therefore be applied in numerous applications for enhancement of consumer properties. For instance, 2-ethyl-3-methylpyrazine (**23**) is used as an additive in bread, popcorn, peanut products, 2,3,5-trimethylpyrazine (**9**) is used to enrich chocolate flavour and 2-ethyl-3-methoxypyrazine (**67**, Figure 6) is often used to heighten the potato flavour of dehydrated potatoes in for example potato salad.[6, 32]

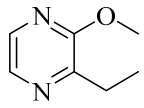
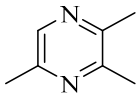
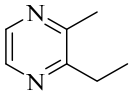
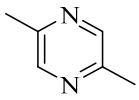
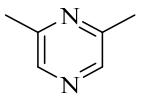
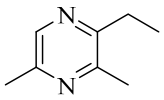
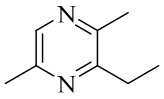
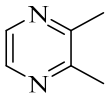
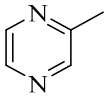
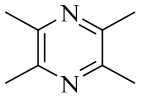
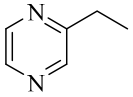


Figure 6. The structure of 2-ethyl-3-methoxypyrazine (**67**).

It is also common to add various pyrazine derivatives to microwave food since the roasty odour compounds do not form below 200 °C and tend to only develop with prolonged heating as suggested by Yeo. *et al* 1991, hence are not naturally produced through rapid microwave heating.[5, 33]

Table 6. The detection of some pyrazine derivatives, usage per annum and quantitative occurrence in foods.[32]

Pyrazines	No.	Reported as additive to listed foods
	**9**	Boiled beef, cocoa products, and roasted peanuts
	**23**	Coffee, roasted barely, and roasted peanuts
	**24**	Heated beef fat, casein, and cocoa products

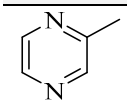
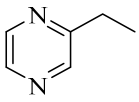
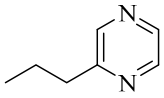
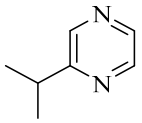
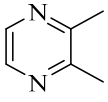
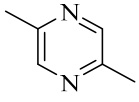
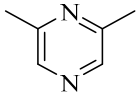
	**25**	Spray dried whey, tomatoes, and potato chips
	**1**	Cocoa products, potato chips and hydrolysed soy protein
	**26**	Boiled beef, rye crisp bread and coffee
	**27**	Roasted beef drippings and peanuts
	**28**	Casein, tomatoes and roasted pecans
	**29**	Coffee, lactose-casein, and natto
	**30**	Cocoa products, coffee, potato chips

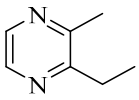
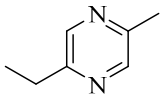
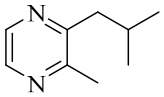
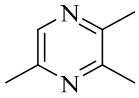
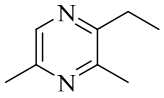
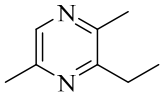
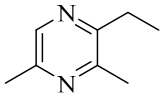
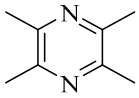
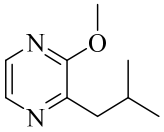
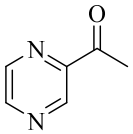
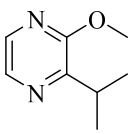
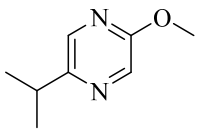
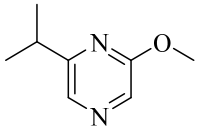
As well as naturally isolated species, chemically synthesised flavours have gained prevalence in our foods, initially due to the increasing global demand for key flavoured products such as strawberry and vanilla, but also as a result of the development of organic chemistry.[6] To evaluate the intake level of flavouring ingredients in case of posing risks to consumer, there are several monitoring organisations undertaking studies and publishing comprehensive lists of chemically defined flavouring groups which have become standards.[3, 17] Since toxicological concerns such as acute, subchronic and chronic impact are crucial to immediate human health these organisations have significant power and responsibility. The current evaluations of flavouring substances are undertaken by:

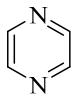
1. the FEMA (Flavour and Extract Manufactures Association) GRAS (Generally Recognised as Safe) program.
2. the joint FAO/ WHO Expert Committee on Food Additives (JECFA) evaluation of flavouring groups.
3. EFSA flavouring group evaluations.

For example, the FEMA panel has re-evaluated more than 1700 GRAS flavouring substances under conditions of intended use since 1992.[3] The assessments of pyrazine compounds used as flavour ingredients was as such comprehensively reviewed in 2002.[3] In this review, the exposure, structural analogy, metabolism, pharmacokinetics and toxicology of 41 commonly used pyrazines were summarised. While the toxicity studies were carried out in many orders of magnitude greater than actual intake levels and the consumption level of pyrazines from natural sources is much greater than the intended addition as flavouring substances, the group of pyrazines in this report was determined to be GRAS (generally recognised as safe).[3] Table 7 lists some examples of the assessed 41 pyrazine derivatives where all the listed pyrazines were reported with a consumption ratio greater than 1 (i.e., the predominant intake of the pyrazine derivative is from food).

Table 7. Identity and exposure data for pyrazine derivatives with consumption ratio greater than 1.[3]

Flavouring ingredient	No.	FEMA registration No.	Annual consumption volume (kg)
	**28**	3309	50
	**30**	3281	44
	**31**	3961	1
	**32**	3940	1
	**27**	3271	27
	**24**	3272	59
	**25**	3273	18

	**23**	3155	72
	**33**	3154	6
	**34**	3133	0.05
	**9**	3244	347
	**1**	3149 (for **1** and **26**)	72 (for **1** and **26**)
	**26**		
	**1**	3150	2
	**29**	3237	144
	**21**	3132	7
	**35**	3126	923
	**20**		
	**36**	3358 (for **20**, **36** and **37**)	0.5 (for **20**, **36** and **37**)
	**37**		

	**38**	-	1.2

Prevalence and usage in wines

Methoxypyrazines are generally suggested to biosynthetically originate in grapes (*Vitis vinifera*) at the pre-ripening stage which become predominantly metabolised or volatised during ripening.[34] These have been found in *Vitis vinifera* and in commodity products like wines made from *Vitis vinifera*. The most abundant methoxypyrazines detected for 'wine aroma' are 2-isopropryl-3-methoxypyrazine (**20**), 2-isobutyl-3-methxoypyrazine (**21**), and 2-*sec*-butyl-3-methoxypyrazine (**22**), while 2-methoxypyrazine (**65**), 2-methoxy-3-methylpyrazine (**66**), 2-methoxy-3-ethylpyrazine (**67**), and 2-methoxy-3,6-dimethylpyrazine (**68**) were detected.[35]

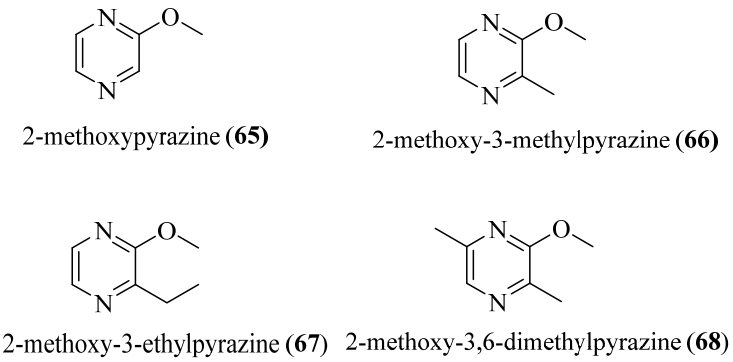


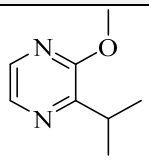
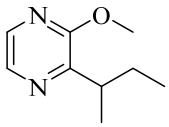
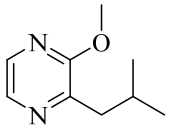
Figure 7. The structures of methoxypyrazine found in wine.

A list of olfactory thresholds methoxypyrazines **20**-**22** in various media were summarised by Sidhu *et al.* in 2015, (Table 8).[36] Among all the MPs listed in Table 8, compound **22** has the highest concentration in grapes and is proposed to be responsible for the distinct vegetable character of Cabernet Sauvignon wines.[34] Subsequently, compound **20** was detected in *V. vinifera* which is more commonly found in green peas (3500 ng L^{-1}). Whilst compound **22** is even less abundant in *V. vinifera* but more concentrated in beetroot (5600 ng L^{-1}).[34]

The presence of compound **21** was found to correlate with the genotype of the fruit by grafting experiment which further confirms its internal biosynthesis rather than translocation from vegetative tissue.[37] In addition, compound **20** levels can also be

enhanced by a common (Australia) vineyard pest known as the "multi-coloured Asian lady beetle" (*Harmonia axyridis*) or seven-spot ladybeetle (*Coccinella septempunctata*).[38] The bug-related MP increase leads to a complex off-odour problem in the affected juice and derived wines, namely, the "ladybug taint" as commonly described in the literature.[39]

Table 8. The odour description and olfactory thresholds of three methoxypyrazines in water (Adapted from Sidhu *et al*. 2014).[36]

Entry	Methoxy pyrazine	Odour description	Olfactory Thresholds / ng L^{-1}	Ref.
1	 2-isopropyl-3-methxoypyrazine (**20**)	Galbanum, earthy, musty, potato, green pepper, roasted, peas, moldy, cellar	1 - 2	[1, 36, 40-42]
2	 2-*sec*-butyl-3-methoxypyrazine (**22**)	Green (peas, bell pepper, galbanum), ivy leaves, bell pepper	1 – 2	[42,22, 41, 43,4]
3	 2-isobutyl-3-methoxypyrazine (**21**)	Green, bell pepper, musty, earthy	10 2	[42] [44,1, 36, 41]

Presence and application in Fragrances

In addition to their use as aesthetic flavouring substances, pyrazines are also extensively used in fragrances and toiletries. Both their odour character and chemical stability (aromatic stability) make pyrazines excellent components for fine perfume but also for more robust cleaning products where the chemical formulation (high/low pH or oxidation potential) can be more detrimental to other more sensitive molecules. The

odour descriptions for these pyrazines are widely encompassing generating fresh, green, woody, ambery, oriental, musky, minty, and herbaceous notes. Some examples of pyrazines and their occurrence in fragrances has been reviewed and summarised by Zviely in 2010 (Table 9).[10] Zviely also provided a demonstration formula of petitgrain (a Citrus aurantium blend which is considered a brilliant for reminding people of spring and chasing away the January's Winter Blues!) where 2-isobutyl-3-methoxypyrazine (**21**) is noted as one of the ingredients (Table 9).

Table 9. Examples of pyrazine compounds used in fragrance.[10]

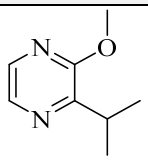
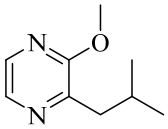
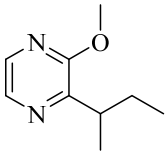
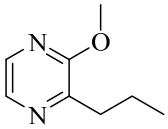
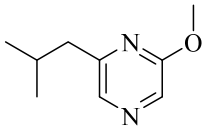
Entry	Pyrazine structure	Pyrazines and their applications in fragrance
1		2-isopropyl-3-methoxypyrazine (**20**) → "Applied as a trace component to green compounds for all purposes."
2		2-isobutyl-3-methoxypyrazine (**21**) → "Applied in traces to green-floral compounds, e.g., petitgrain."
3		2-*sec*-butyl-3-methoxypyrazine (**22**) → "Applied as trace component of chyprefloral - animalic concept perfumes."
4		2-*n*-propyl-3-methxoypyrazine (**39**) → "Applied as trace component to green compounds for all purposes."
5		6-isobutyl-2-methoxypyrazine (**40**) → "Applied in traces to green-floral compounds for all purposes."

Table 10. The demonstration formula of petitgrain involving 2-isobutyl-3-methoxypyrazine (**21**). Adapted from Zviely in 2010.[10]

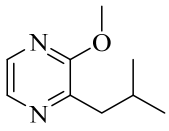
Ingredient	Composition/Parts
Methyl anthranilate	2

Clary sage oil	5
Caryophylene	5
Nerol	8
Neryl acetate	10
Citronellol	15
Orange terpenes	25
Terpenyl acetate	30
Geraniol	40
α-Pinene	40
Geranyl acetate	60
Terpineol	100
Linalool	250
Linalyl acetate	400
2-Methoxy-3-isobutylpyrazine	10

1.2.2 Natural occurrence and biological activities of pyrazines in prey-predator relationship

Chemical communication with various odour notes is recognised between prey and predators in a predation sequence (i.e., detection, identification, approach, subjugation, and consumption). These semiochemicals, in other words, behaviour-controlling chemicals are secreted by organisms to regulate their social and sexual interactions.[27] This odour communication often works in conjunction with other signals including taste, visualization (of specific colouration, behavioural displays) and audition modality. Pyrazines have been identified as one of the semiochemical signal molecules used by species of bacteria, insects, mammals, vertebrates, and plants.[29, 31]

The aposematic tiger wood moth (*Arctia plantaginis*) is a well-studied example of an insect producing a pyrazine containing secretion as a chemical alert.[45,46] Specifically, two methoxy pyrazines, 2-*sec*-butyl-3-methxoypyrazine (**22**) and 2-isobutyl-3-methxoypyraizne (**21**) have been found in the defensive neck fluid of *A. plantaginis* (Figure 8).



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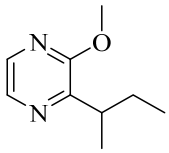
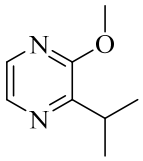


22

Figure 8. The picture of a tiger moth. Photo 205405077 © Jason W. Baker | Dreamstime.com.

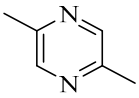
This acts as protection for the moth from bird attack which also induces predator learning when combined with visual alerting signal (the predominant warning markings).[46] The related compound 2-isopropyl-3-methoxypyrazine (**20**) has been identified as a distinctive odour character of the seven-spot ladybird beetle (*C. septempunctata*, Figure 9).[27] In such coccinellid species the compound has been suggested to possess a dual function firstly as an alerting signal but also acting as a species aggregation pheromone.



20

Figure 9. The picture of a seven-spot ladybird on flower. Photo 192664103 © Martin Hatch | Dreamstime.com.

Two alkylpyrazines, 2,5-dimethylpyrazine (**24**) and 3-ethyl-2,5-dimethylpyrazine (**26**) have been found to be used by ants (*Atta Sexden*, Figure 10) as trail pheromones and alarm pheromones for the presence of other ants (note: pheromones are semiochemicals used for conspecific communication, whilst allelochemicals are responsible for interspecific communication[29]).[26]



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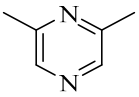

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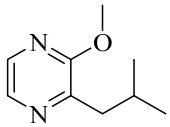
Figure 10. A picture of a species of leafcutter ant with associated pyrazine sent molecules. Photo 23098982 © Nick Vermeulen , Dreamstime.com.

The compound 2-isobutyl-3-methoxypyrazine (**21**) has also been isolated from certain species of tree frogs (*H. pulchellus*, Figure 11).[47] In 2017, the first fluorescent frog (*Hypsiboas punctatus*) was reported by Nowogrodzki *et al.* where longer wavelength is re-emitted than absorbed.[48] The brightness of fluorescent molecules is equivalent to 18% of visible light at full moon. This finding was suggested to be promising in exploring the ecological and behavioural function of fluorescence.[48] This area is also potentially a massively untapped biological source which may help to develop novel pharmaceuticals.[47] Research has been designed to analyse and test synthetic and naturally occurring components from predators on the response of prey species. This is a large research area centred around the impact of such regulating compounds on animal and pest behavioural modification, for example on crop protection from insect consumption to pet health and wellbeing.



21

Figure 11. A picture of a species of a green tree frog with associated pyrazine sent molecule. Photo 5573258 © Heysues23 | Dreamstime.com.

A collection of synthetic and naturally occurring pyrazines has been compared to analyse the function and variance on the influence of prey and predator relationship. For instance, 2,5-dimethylpyrazine (**24**), 2,6-dimethylpyrazine (**25**), 2,3,5-

trimethylpyrazine (**9**) and 2,5-dimethyl-3-ethylpyrazine (**7**) are known kairomones released by the grey wolf (*Canis lupus*) which initiate fear-related behaviour in both rodents and ungulates such as mice and deer (Figure 12).[29] Since excessively large population of deer may negatively affect agriculture, horticulture and forests, repellents can be humanely used to protect these resources. The natural, non-carcinogenic and low acute toxicity characters also made the pyrazine cocktail more environmentally benign. The high repellent function of a natural pyrazine containing allelochemical cocktail on deer has been confirmed and gave lasting effects of at least one month from application.[49] In a similar way synthetically derived odours have shown various influential ability as repellents to species, an area which is seeing rapid development.[50]

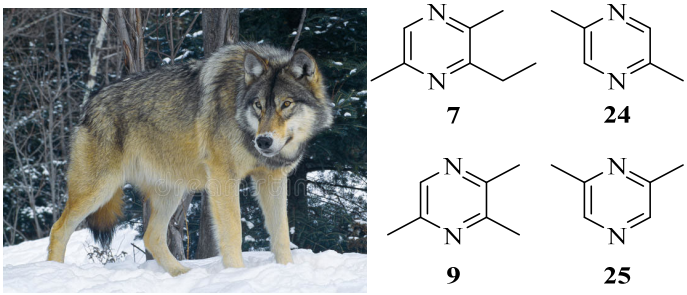


Figure 12. A picture of a grey wolf with associated pyrazine sent molecules. Photo 5573258 © Heysues23 | Dreamstime.com.

1.3 Pyrazine formation

The change in food flavour after prolonged heating has attracted a great deal of research attention towards the analysis and identification of key flavour and odour chemicals and their preparation.

1.3.1 Pyrazine formation in heated food - The Maillard Model system

Many pyrazine derivatives possess a roasty aroma and are isolated from foods after thermal process such as frying and roasting. Carbohydrates are degraded and processed with amino acids in a series of pathways involving the Maillard Reaction. The name is obtained from the French chemist Louis Maillard, who first described the transformations of saccharides and amino compound in 1910.[8] The main factors affecting the pathway followed and thus the specific aroma developed in Maillard reactions are: identities (structures) of the sugar and amino acids, pH, the reaction

temperature, and water content, mainly affecting the kinetics of reactions.[51]

The essential steps of the Maillard Reaction have been divided into three phases: the early, advanced, and final phases. Pyrazine derivatives are formed from the aminoketones generated during the advanced stage of the Maillard Reaction and procedures include: Strecker degradation, condensation of amino ketones to form dihydropyrazine derivatives followed by oxidation reaction to obtain the corresponding pyrazine derivatives (Figure 13).[52] The reaction mechanism of pyrazine formation in Maillard Reaction is shown in Scheme 1 and Scheme 2.

Strecker degradation (Scheme 1):

Initially, the dicarbonyl compound **a** (Figure 13), was obtained from sugar degradation via Amodori rearrangement product and reacts with an amino acid **b**.[52] After losing a hydronium from **c**, decarboxylation occurs through **d** followed by enol-keto tautomerisation (**e**→**f**). Hydrolysis of the intermediate imine **f** forms the key α-aminoketone pyrazine precursor **h**.

Condensation of aminocarbonyl compounds (Scheme 2):

The secondary transformation involves condensation of two α-aminocarbonyl compounds **h** and **h'** to form a dihydropyrazine **k** as shown in Scheme 2. To obtain the desired pyrazines, two pathways were proposed, and either one is possible. If the dihydropyrazine **k** oxidises spontaneously, pyrazine **r** will be produced via pathway **A**. Alternatively, the dihydropyrazine anion **o** could react with another Strecker aldehyde **i** in an aldol-type reaction to form pyrazine **m** (pathway **B**).

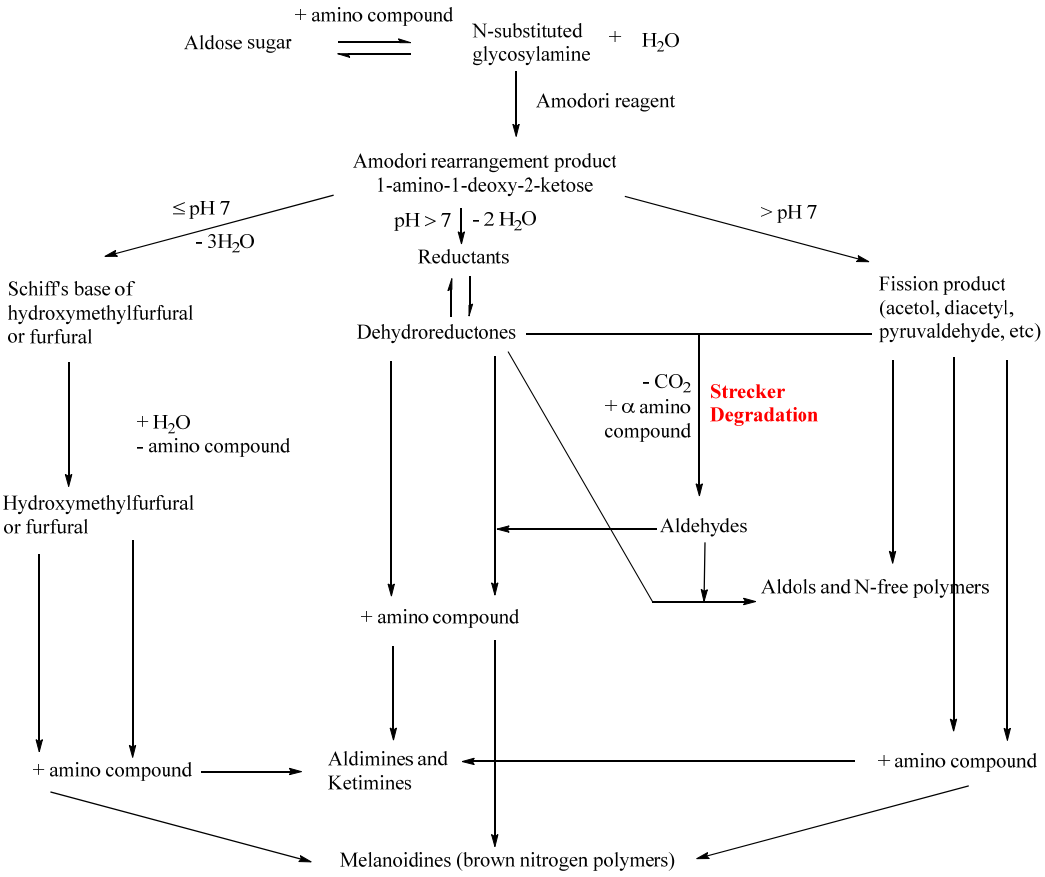
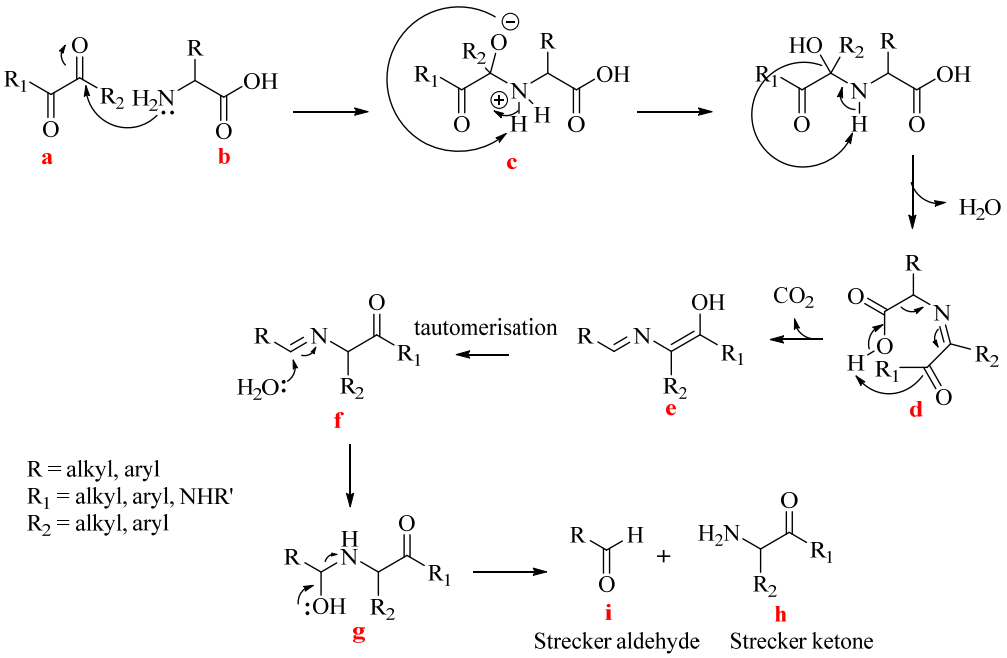
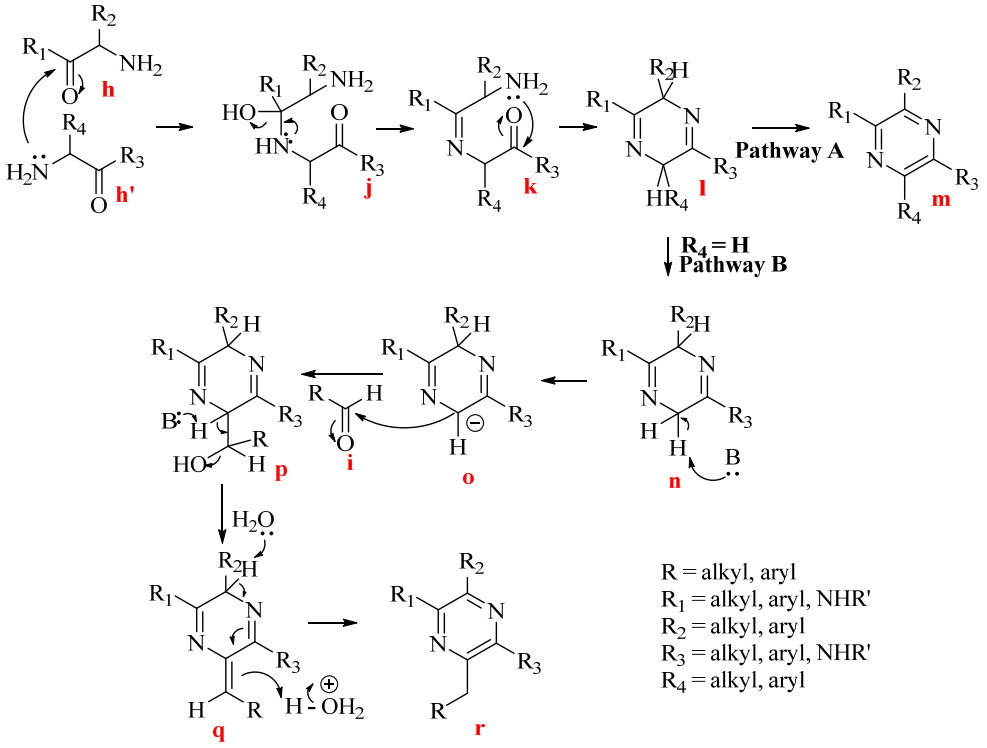


Figure 13. Maillard reaction schemes.[8]



Scheme 1. General mechanism of Strecker degradation to form precursors of pyrazines.[53]



Scheme 2. The formation of dihydropyrazine **p** after Strecker degradation and two pathways to form pyrazine derivatives **m** and **r**.[53]

1.3.2 Formation in heated food, pyrazines can be synthesised through fermentation process in food and beverage such as *baijiu and Natto.* (Figure 14).

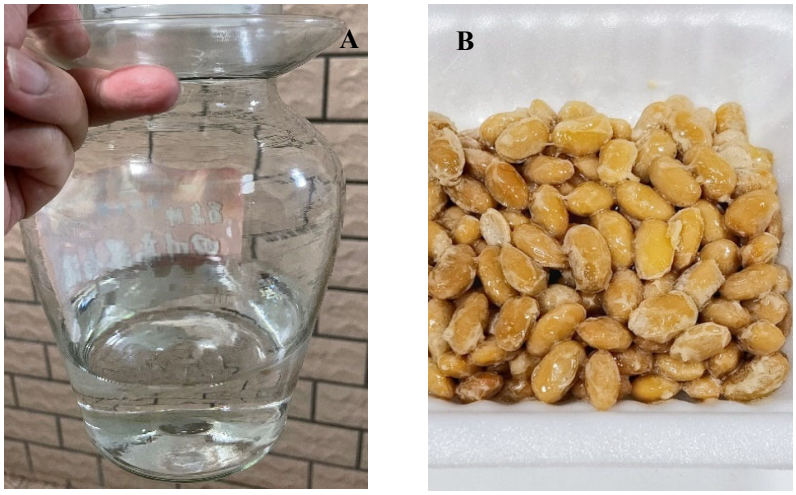


Figure 14. A. The picture of *Baijiu*, a type of alcoholic beverages from China. **B**. The picture of *Natto*, a type of fermented soyabeans from Japan.

The strain that is responsible for this biosynthetic pathway is *Bacillus subtilis*, a GRAS (generally regarded as safe) organism.[54] It was recognised to assist the formation of pyrazine precursors (such as α-acetolactate, acetoin, free amino acids and ammonia) rather than the desired alkylpyrazines through metabolic activities.[55]

In a biosynthesis mechanism study, 2-ethyl-3,5-dimethylpyrazine (**1**) and 2-ethyl-3,6-dimethylpyrazine (**26**) were found to be present in high concentration in samples of isolated *Bacillus subtilis* strain from Chinese baijiu under aerobic condition.[54] Another study also confirmed the contribution of 2-ethyl-3,5-dimethylpyrazine (**1**) to the key aroma profile of Chinese Zhima aroma-type baijiu, which is fermented from grain under an open aerobic environment.[56]

Zhang *et al*. 2020 [54] proposed a biosynthesis pathway based on initial isotopic tracing result (Figure 15). In this pathway, compounds **1** and **26** were produced from *L*-threonine and *D*-glucose at environmental temperature and pressure. The metabolization of *L*-threonine and *D*-glucose produce aminoacetone and 2,3-petadione respectively which condense together to form the dihydropyrazine derivatives **47** and **48**. These dihydropyrazine derivatives are subsequently oxidised to form the desired

aromatic pyrazines **1** and **7**.[54]

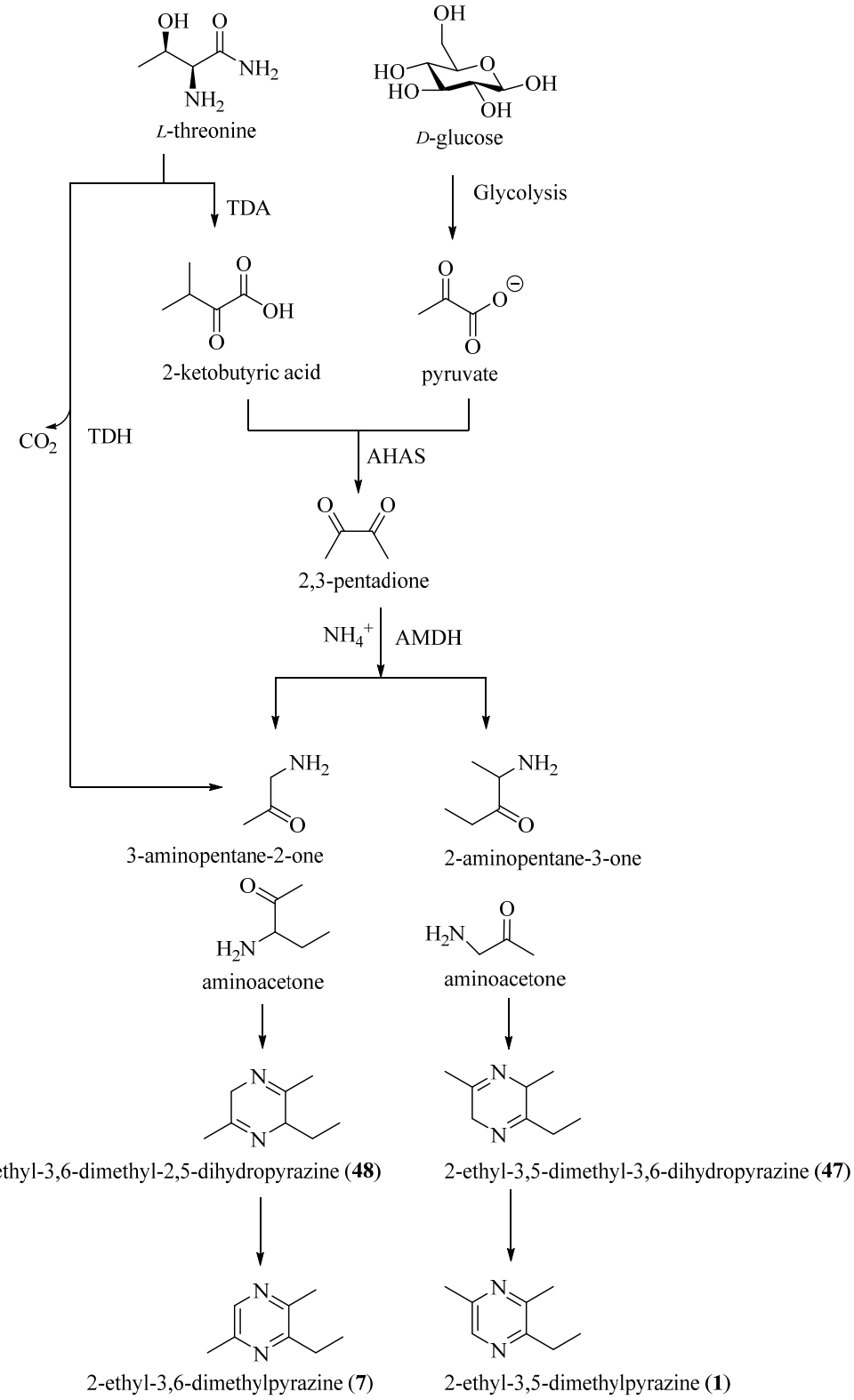


Figure 15. The proposed biosynthetic pathway for 2-ethyl-3,5-dimethylpyrazine (**1**) and 2-ethyl-3,6-

dimethylpyrazine (**7**) with *Bacillus subtilis*. TDH: *L*-theronine-3-dehydrogenase; TDA: *L*-threonine deaminase; AMDH: amine dehydrogenase.[54]

Two biosynthetic pathways have been proposed for the generation of 2-methoxy-3-alkylpyrazines.[34,22, 35,57] The first pathway involves amidation of an amino acid (Figure 16a). This is followed by condensation with an α,β-dicarbonyl compounds such as glyoxal or glyoxylic acid to form a hydroxypyrazine. In the final methylation step, *S*-adenosyl-*L*-methionine (SAM) serves as the methyl donor. The SAM dependent enzyme for *O*-methylation in *V. vinifera* grapes is known as *O*-methyltransferase (OMT).[58]. In addition, the OMT genes were investigated by Dunlevy *et al*. and subsequently named as *V. vinifera* OMTs (VvOMTs).[37] Four OMT genes were isolated so far from grape genes with different catalytic activity. All of them are capable of methylating hydroxypyrazines forming methoxypyrazines. The second pathway suggested by Cheng *et al*. 1991 involves condensation of two amino acids (valine and glycine) followed by the methylation and the oxidation step to form methoxypyrazines (Figure 16b).[57,59]

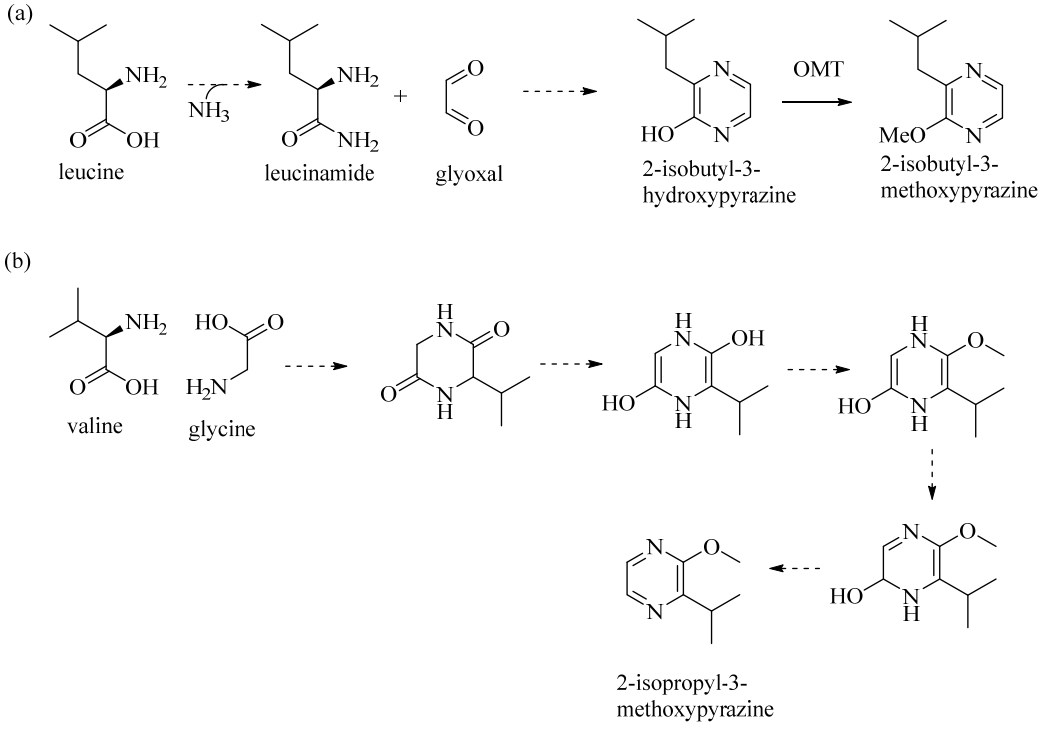


Figure 16. Two proposed biosynthetic pathways for 2-alkyl-3-methoxypyrazine (**21**). OMT: *O*-methyltransferase. (a) Biosynthesis with leucine and glyoxal proposed by Murray *et al.* 1970;[22] (b) Biosynthesis with valine and glycine proposed by Cheng *et al*. 1991.[57, 59] No biochemical evidence has

confirmed the pathway with dashed arrows.[35]

As can be seen pyrazines are a diverse and valuable heterocyclic framework which contribute to many areas of biology and have been used to enhance human society. The prospect is for these compounds to gain increasing utilisation as more is understood about their potential wider usage as semiochemicals and steady growth in usage in the fragrance and flavours industry. As such new preparative methods will be needed to generate new derivatives with different profiles as well as being able to scale up the syntheses of known structures, this is a fertile area for research.

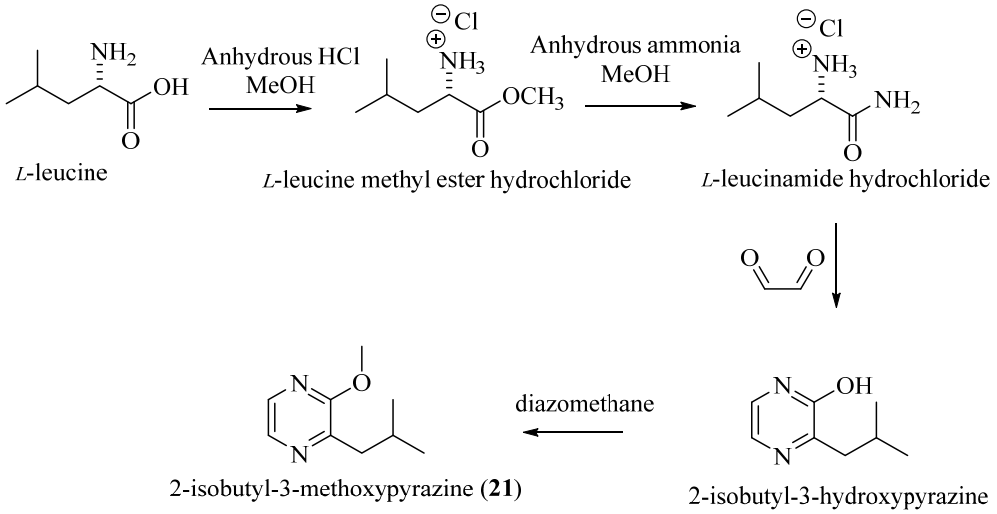
2. Results and Discussion

Overall aims of the project:

1. To improve upon the literature preparation of 2-isobutyl-3-methxoypyrazine (**21**) as first reported by Seifert *et al*., 1970 [1] using new chemical developments.
2. Apply the synthetic procedure developed in Aim 1 to two other methoxypyrazine derivatives, namely: 2-isopropyl-3-methoxypyrazine (**20**) and 2-*sec*-butyl-3-methoxypyrazine (**22**) both of which are important commercial targets.
3. Attempt the preparation of 2-isobutyl-3-methoxypyrazine (**21**) through condensation of 1,2-dicarbonyl and diamine compounds via the intermediate dihydropyrazine derivative which will be evaluated for oxidative aromatization.

2.1 Preparation of 2-isobutyl-3-methoxypyrazine (21)

In 1969, Buttery *et al*. extracted 2-isobutyl-3-methoxypyrazine (**21**) from bell pepper and synthetically prepared this compound in 4 synthetic steps from amino acid, *L*-leucine (Scheme 3). The reaction involves esterification, amide formation, condensation of 1,2-dicarbonyl compound (glyoxal) with the diamine compound (*L*-leucinamide hydrochloride) to form 2-isobutyl-3-hydroxypyrazine and finally methylation to afford 2-isobutyl-3-methxoypyrazine (**21**).

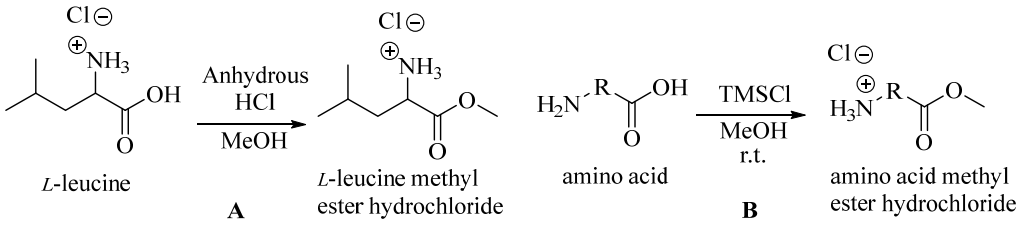


Scheme 3. The synthetic pathways of making 2-isobutyl-3-methoxypyrazine (**21**) as proposed by Seifert *et al*. in 1970.[1]

Our aim was to investigate this simple paper-based synthesis and evaluate its facility for scaling and use in derivative preparation.

2.1.1 Preparation of *L*-leucine methyl ester hydrochloride

Originally, the esterification of the amino acid used methanol and anhydrous HCl as an acid catalyst.[44] There are several literature protocols for conducting the esterification by generating the acid *in situ* i.e., from thionyl chloride or trimethylchlorosilane or through the addition of dehydrating agents like 2,2-dimethoxypropane or adding ion-exchange resins as the promoting acid source. Considering additional aspects such as safety, waste disposal and energy consumption as part of the synthesis, the procedure published by Li *et al*. 2008 was followed.[60] In this synthesis, two equivalents of trimethylchlorosilane in methanol were used to syntheses a range of amino acid ester hydrochloride salts at room temperature. Upon completion of synthesis, the solvent was removed under reduced pressure to afford the desired *L*-leucine methyl ester hydrochloride which could be used directly in the subsequent step.[60]



Scheme 4. A. The original synthesis of amino acid methyl ester hydrochloride with anhydrous HCl in methanol proposed by Seifert *et al*. 1970. **B**. The general synthesis of amino acid methyl ester hydrochloride with trimethylchlorosilane in methanol.

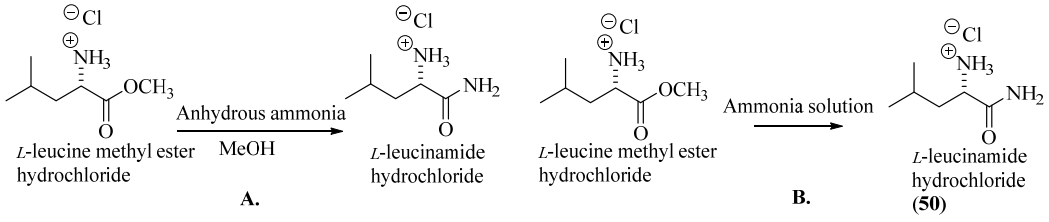
This method was successfully applied to generate *L*-leucine methyl ester hydrochloride (**49**). Two minor observations were the critical need for room temperature and the concentration used in the reaction (Table 11). The dilution of the reaction mixture resulted in a 37% decrease in yield as shown by Entry 1 and Entry 2. We also found that at the laboratory temperature in January in the UK (~8-10 °C), 93% yield was obtained but this required a doubling of the reaction time compared to the literature citation.

Table 11. Reaction results for synthesis in Scheme 4.

Entry	Concentration/ mol dm^{-3}	Time / h	Temperature	Yield/ %
Li *et al*. 2008	1	12	Room temperature	96
1	1	24	Room temperature (< 25 °C)	93
2	0.2	24	Room temperature (< 25 °C)	59
3	1	12	Room temperature (~15 °C)	51

2.1.2 Preparation of *L*-leucinamide hydrochloride

To syntheses *L*-leucinamide hydrochloride (**50**), *L*-leucine methyl ester hydrochloride was treated with anhydrous ammonia gas in methanol in the literature.[1] Later research used ammonia gas, ammonia solution of different concentrations and methanolic ammonia as alternative reagents.[61-62,4,1,63-64]



Scheme 5. A. The original synthesis of *L*-leucinamide hydrochloride (**50**) with anhydrous ammonia in methanol proposed by Seifert *et al*. 1970. B. The synthesis of *L*-leucinamide hydrochloride (**50**) from *L*-

leucine methyl ester hydrochloride and ammonia solution 35%.

Since a solution of ammonia is generally safer to handle than ammonia gas, initially, we followed the method by Oterval *et al*. 2019 (Scheme 5B) using aqueous ammonia solution to synthesis *L*-leucinamide hydrochloride.[64] The initial ammonia solution, 28% was used instead of the specified 25% due to availability in our laboratory, while the difference in the density of these two solutions is 0.01 g cm^{-3}. On average, 20% of *L*-leucinamide hydrochloride was obtained; when the equivalents of ammonia solution was doubled compared to the stated literature amount, and the reaction time was also doubled the yield obtained was less than a third on average (Entry 1, 2, 3 Table **12**).

Table 12. Optimisation studies on the synthesis results of Scheme 5B.[64, 63]

Entry	Equivalents of ammonia source	Ammonia source	Temp/ °C	Time/ h	Yield/ %
Otevrel *et al*. 2019	11	NH_3 (aq), 25%	r.t.	24	72
Gong *et al.* 2014	6 - 7	NH_3 (aq), 25% - 28%	60	6	80
1	13	NH_3 (aq), 28%	r.t.	92	15
2	20	NH_3 (aq), 28%	r.t.	65	< 29
3	26	NH_3 (aq), 28%	r.t.	45	18
4	8	NH_3 (aq), 35%	55	6	89
5	42	NH_3 7 M in MeOH	r.t.	67	-
6	42	NH_3 7 M in MeOH	25	18	-
7	42	NH_3 7 M in MeOH	60	6	-
8	42	NH_3 7 M in MeOH	70	6	-
9	1	Ammonium carbonate	r.t	93	-
10	2	hexamethyldisilazane	40	30	-

Since adjustment of the ammonia solution equivalents and reaction time did not result in improved yield, other ammonia sources were also tested for this procedure. Commercially available methanolic ammonia, 7 M, Alfar Aesar,[61] ammonium

carbonate and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) were screened, however, none of these gave clean conversion as evidenced by TLC analysis compared to the known retention value (R_f = 0.6 in solvent system of DCM:MeOH = 9:1) of *L*-leucinamide hydrochloride (Entry 6-11).

The temperature parameter was then monitored for this reaction and the procedure by Gong *et al*. 2014 was followed as the reference. In this procedure the solution was gently warmed (55 °C) in a closed system (balloon) to help maintained the ammonia pressure as shown in Figure 17. The optimum yield obtained was 89%.

Figure 17. The set-up of the procedure to synthesis *L*-leucinamide hydrochloride. The condenser is stoppered with a septum and a nitrogen balloon on the top to form a closed system with controlled building pressure.

In addition, colourless crystals formed upon standing the reaction at room temperature after three days which was found to be the residual amino acid (Figure 18). As such the unreacted amino could be easily isolated by filtration and even recycled. Cooling cycles could be used to shorten this time period but due to restricted access to the laboratory this was not explored.

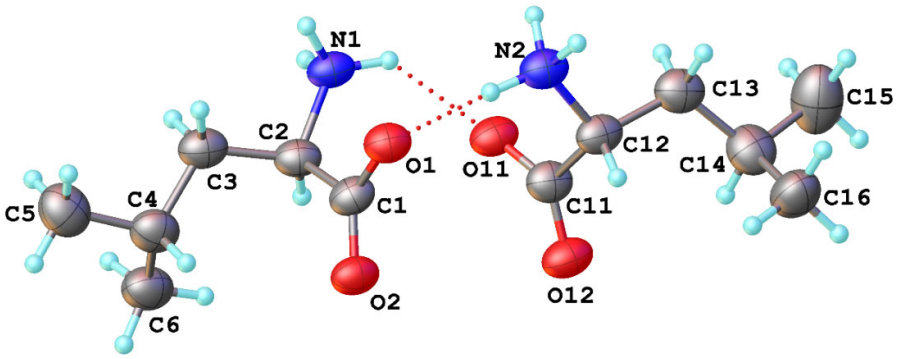
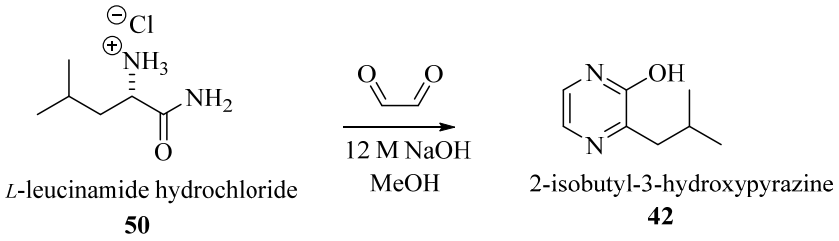


Figure 18. The crystal structure of the colourless crystals confirmed as leucine crystals.

Carefully observing the reaction, it was noted that during the first two hours of reaction, the size of nitrogen balloon gradually expands which cause a limitation of this procedure. The maximum scale that we can perform without balloon rupture was 55 mmol of *L*-leucine methyl ester hydrochloride. Since the *L*-leucine methyl ester hydrochloride was not completely dissolved in ammonia solution, 35%, increasing the pressure in a closed system in a flow machine was also not realised.

2.1.3 Preparation of 2-isobutyl-3-hydroxypyrazine

The condensation of *L*-leucinamide hydrochloride and glyoxal afford 2-isobutyl-3-hydroxypyrazine (**42**) via 6-*exo-trig* ring formation under alkaline condition (Scheme 6).[65]



L-leucinamide hydrochloride
50

2-isobutyl-3-hydroxypyrazine
42

Scheme 6. The synthesis of 2-isobutyl-3-hydroxypyrazine (**42**) from *L*-leucinamide hydrochloride and glyoxal under alkaline condition.[65]

The parameters of pH, equivalents of glyoxal and cooling bath temperature were explored to find optimum conditions for 2-isobutyl-3-hydroxypyrazine (**42**) formation (Table 13). As seen entry 6 had a higher yield than Entry 5 while the reaction time has

been increased by more than two folds. Entry 6 also had a higher yield than Entry 4 when the cooling bath had a lower reaction temperature of 0 °C while adding the reagents. Equivalent yield was obtained for Entry 1 and Entry 5 in which the equivalent of glyoxal were increased from 1.2 to 4.4. Switching solvents from methanol to toluene or acetone did not give improved yields. Overall, longer reaction time and lower cooling bath temperature at around pH 10 in methanol solvent is recommended from the results.

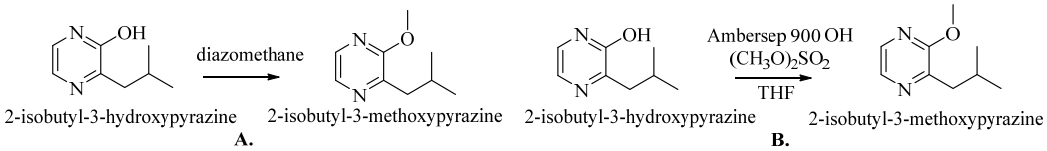
Table 13. The reaction attempts of 2-isobutyl-3-hydroxypyrazine (**51**) of Scheme 6.

Entry	Scale/ mmol	Glyoxal equiv.	Solvent	Temp/ °C[a]	pH	Time/ h	Yield/ %
1	9	1.2	MeOH	-18 to rt	11	5.5	60
2	9	1.3	Toluene	rt to 40	11	3.8	-
3	9	1.5	Acetone	rt to rt	-	Overnight	43
4	10	4.4	MeOH	0 to rt	10	18	63
5	10	4.4	MeOH	-35 to rt	9	7	60
6	10	4.4	MeOH	-35 to rt	10	18	69

[a]rt = 20 °C.

2.1.4 Preparation of 2-isobutyl-3-methoxypyrazine (21) through developed novel synthesis

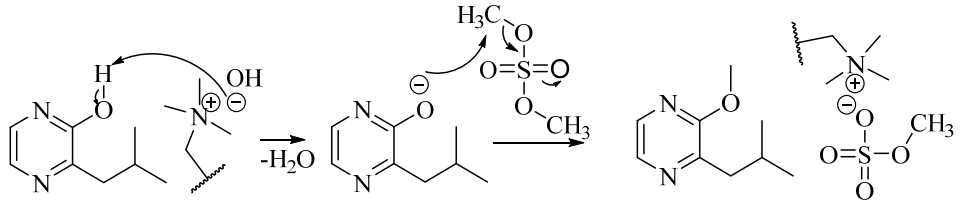
Previously, 2-isobutyl-3-methoxypyrazine (**21**) has been synthesised by methylation of the corresponding 2-isobutyl-3-hydroxypyrzine (**42**) with diazomethane in ether as shown in Scheme 7A.[44] To reduce the risk of handling a highly flammable diazomethane (and ether solvent) at large scale and to simplify the isolation of product, we developed a novel methylation step aided by a quaternary ammonium hydroxide anion exchange resin (Ambersep 900 OH form) as illustrated in Scheme 7B.



Scheme 7. **A**. The synthesis proposed by Buttery *et al*. 1969 for methylation of 2-isobutyl-3-hydroxypyrazine (**42**) to 2-isobutyl-3-methoxypyrazine (**21**). **B**. The novel synthesis of 2-isobutyl-3-methoxypyrazine (**21**) using Ambersep 900 hydroxide form.

In this novel synthesis, the resin acts as a mild base where 2-isobutyl-3-

hydroxypyrazine (**42**) could be sequestered. It can also be regenerated and recycled following the reaction by treatment with sodium hydroxide. The methylation is then completed upon direct addition of dimethyl sulfate to the solution which releases the product from the resin once it forms the methoxy adduct. The solution can then be filtered and concentrated *in vacuo* to afford the desired product. In addition, other impurities such as unreacted reagents are trapped by the resin to save the effort in tedious work procedure. The trapping step is completed within 10 minutes and the subsequent methylation in an hour.



Scheme 8. The reaction mechanism for 2-isobutyl-3-methoxypyrazine (**21**) synthesis as shown in Scheme 7B.

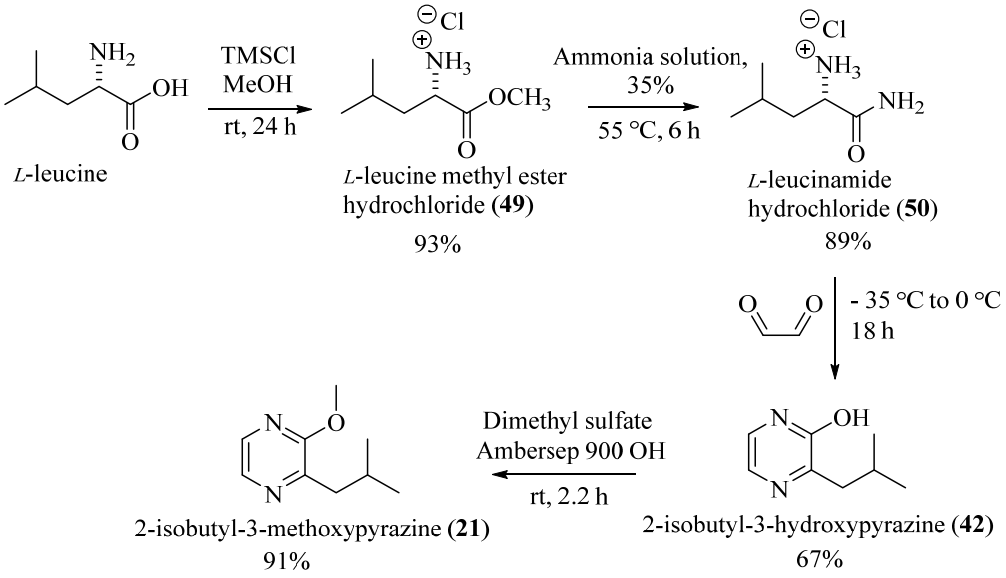
The whole process can be traced by disappearance of starting material spot and the appearance of product spot in solution by thin-layer chromatography. Through the screening process as shown by Table 14, two equivalents of dimethyl sulfate were found to obtain the optimum yield of 2-isobutyl-3-methoxypyrazine (**21**, 91%). In addition, seven grams of Ambersep 900 OH was found to trap 3.3 mmol of 2-isobutyl-3-hydroxypyrazine (**42**) in the first step and the rest of impurities such as excess regents in the second step. Overall, this novel methylation step is performed under ambient conditions, with a simple work up and results in excellent yield of the desired product.

Table 14. The reaction results of 2-isobutyl-3-methxoypyrazine (**21**) of Scheme 7B.

Entry	Scale/ mmol	Equivalents of $(CH_3O)_2SO_2$	Ambersep 900 OH/ g	Yield/ %
1	3.3	0.54/1.3	3	56
2	3.3	0.7	4	54
3	3.3	1.0	5	60
4	3.3	1.5	5	71
5	3.3	1.5	5	72
6	3.3	1.8	6	80

7	3.3	2.0	7	91
8	3.3	2.2	8	86

Hence, the synthesis of 2-isobutyl-3-methoxypyrazine (**21**) is adapted from Scheme 3 to the final reaction process as shown in Scheme 9:

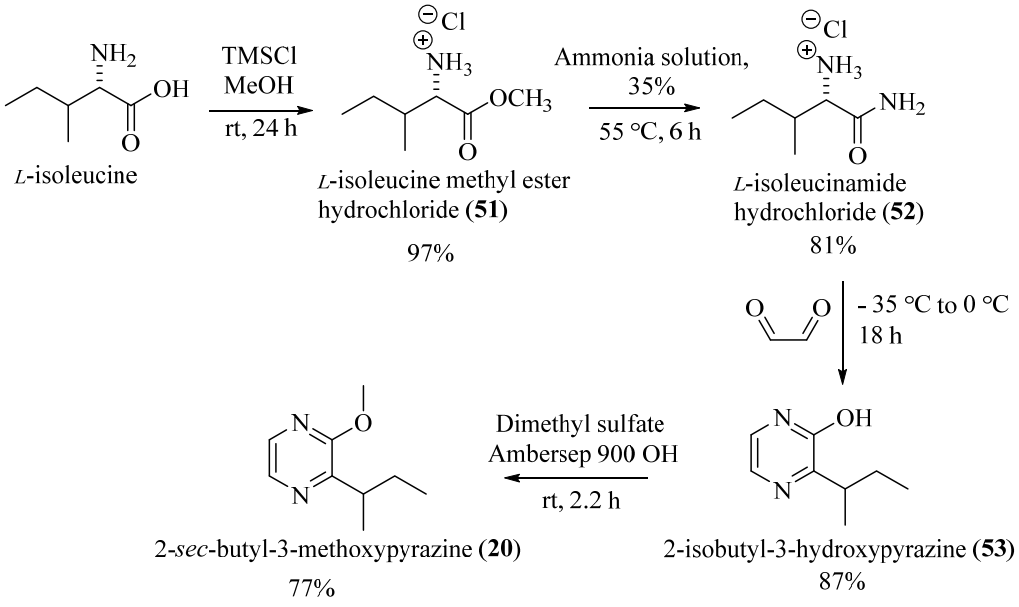


Scheme 9. The overall synthetic pathway of preparing 2-isobutyl-3-methoxypyrazine (**21**).

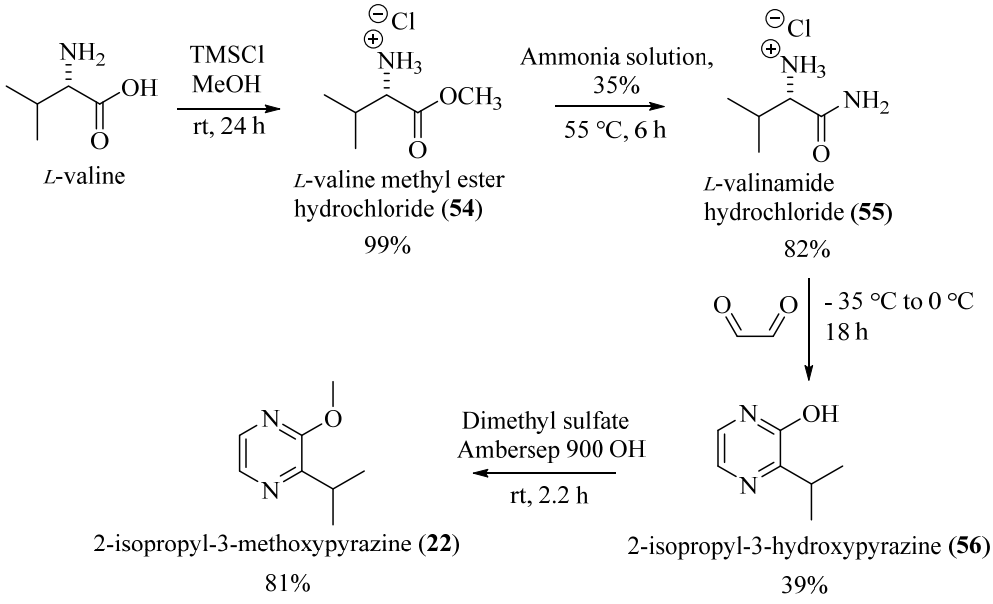
Starting from *L*-leucine this 4-step synthesis accomplished the generation of the desired 2-isobutyl-3-methxoypyrazine (**21**) in an overall yield of 50%. This compares to the original literature in which the compound was prepared by Seifert *et al.*, 1970[1] in less than 19% overall isolated yield.

2.1.5 Synthesis of 2-isopropyl-3-methoxypyrazine (20) and 2-*sec*-butyl-3-methoxypyrazine (22)

Next, we applied the route outlined in Scheme 8 to prepare the derivatives 2-isopropyl-3-methoxypyrazine (**20**) and 2-*sec*-butyl-3-methoxypyrazine (**22**), from amino acids of *L*-valine and *L*-isoleucine respectively. The results are summarised in Scheme 10 and Scheme 11.



Scheme10. The reaction scheme of 2-*sec*-butyl-3-methoxypyrazine (**22**) preparation.



Scheme 11. The reaction scheme of 2-isopropyl-3-methoxypyrazine (**20**) preparation.

In addition, the specific ester chain length generated in the amino acid ester hydrochloride required to perform amidation was noted. The *L*-isoleucine methyl ester hydrochloride and *L*-isoleucine ethyl ester hydrochloride were both tested for the amidation procedure (Figure 18). The *L*-isoleucinamide hydrochloride was obtained easily as the methyl ester but issues with isolation were encountered when attempting to prepare the ethyl ester.

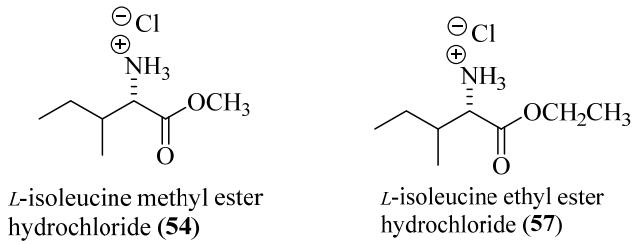
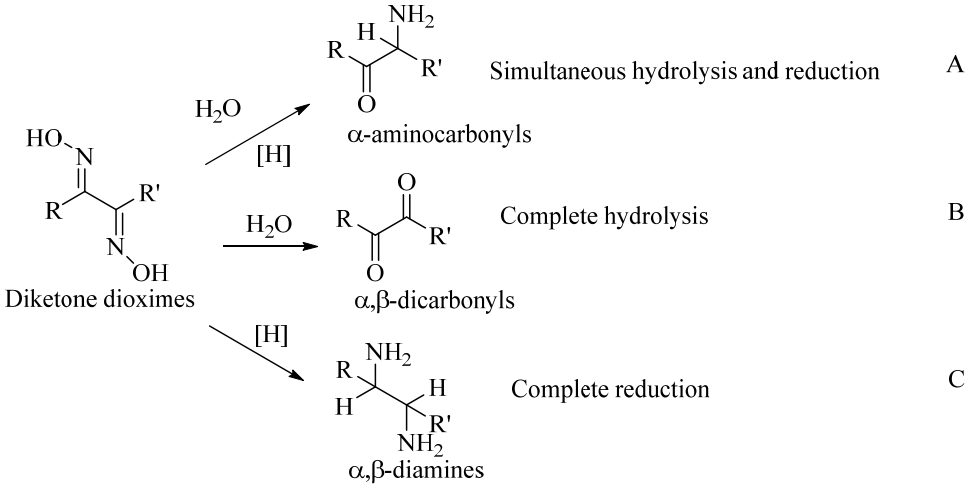


Figure 19. The structure of *L*-isoleucine methyl ester hydrochloride (**54**) and *L*-isoleucine ethyl ester hydrochloride (**57**).

2.2 Preparing 2-isobutyl-3-methoxypyrazines through condensation of α,β-diketone compound and α,β-diamine via 2,3-dihydropyrazines

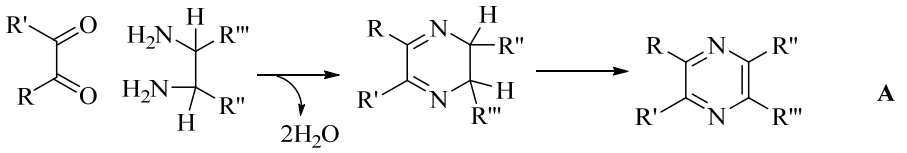
In section 2.1, 2-isobutyl-3-methxoypyrazine (**21**) was prepared via condensation of glyoxal and the previously synthesised aminoamide compound **50**. Alternatively, other diamine derivatives (surrogates of the aminoamide compound **50**) can be commercial sourced such as ethylenediamine and ethylenediamine dihydrochloride, these can be condensed with easily prepared α,β-dicarbonyl compounds. For example, complete hydrolysis of diketone dioximes were suggested to afford good isolated yields of α,β-diketones for pyrazine synthesis (Scheme 12B). Through reduction the same diketone dioximes may also be used to access 1,2-diamines (Scheme 12C) or form α-aminocarbonyls through simultaneous hydrolysis and reduction (Scheme 12A). Hence, pyrazine derivatives were suggested to form either via self-condensation of α-aminocarbonyls, or condensation of α,β-diketones and a 1,2-diamine.



Scheme 12. Diketone dioximes were suggested to undergo: **Route A**. forming α-aminocarbonyl

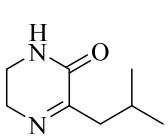
derivatives under simultaneous hydrolysis and reduction; **Route B**. forming diketones after complete hydrolysis; **Route C**. forming diamines via complete reduction.

The condensation of α,β-diketone and 1,2-diamine to form a 4,5-dihydropyrazine followed by oxidation procedure is one of the most widely utilised pyrazine ring syntheses. This approach has been shown to proceed smoothly when R and R' are aryl (Scheme 13A), however, low-molecular weight alkyl derivatives are suggested to favour polymer formation thus giving poor over isolated yields. In 2012, Ghosh and co-workers established one of such procedures under ambient conditions in methanol with added *tert*-butoxide as the catalyst (Scheme 13B).[2] The range of isolated yield for the 18 alkyl pyrazine derivatives prepared in the study was 74 – 88%.

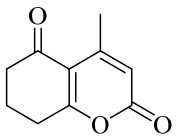


Scheme 13. The preparation of alkyl pyrazines through condensation of α,β-diketone and 1,2-diamine by Ghosh *et al*. 2012.[2]

In our study, we also generated an β-diketone precursor, namely, ethyl 4-methyl-2-oxopentanoate (**58**) for condensation with ethylenediamine to form 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**41**) (Figure 20). The spontaneous oxidation was not observed after isolation instead only the dihydropyrazine was obtained. Interestingly, even in the presence of added catalyst; *tert*-butoxide, in methanol, equivalent conditions to those reported by Ghosh, no evidence of oxidation was found. Alternatively, aromatisation via bromination of the dihydropyrazine derivative **41** was developed from another model dihydro compound **43** since this compound was readily available in laboratory. The detailed reaction scheme can be found in section 2.2.3.1.



3-isobutyl-5,6-dihydropyrazine
-2(*1H*)-one **(41)**

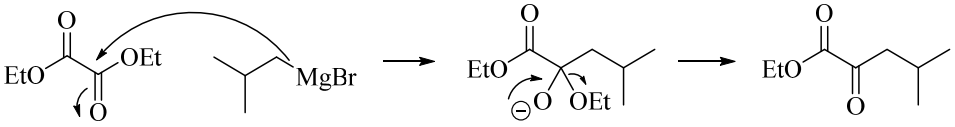


4-methyl-7,8-dihydro-2H-chromene
-2,5(*6H*)-dione **(43)**

Figure 20. The structure of 3-isobutyl-5,6-dihydropyrazine-2(*1H*)-one (**41**) and 4-methyl-7,8-dihydro-2*H*-chromene-2,5(*6H*)-dione (**43**).

2.2.1 Preparation of ethyl 4-methyl-2-oxopentanoate

The β-diketone compound **58** with the required isobutyl substituent was prepared from a Grignard reaction of isobutyl magnesium chloride with diethyl oxalate (Scheme 15).[66]

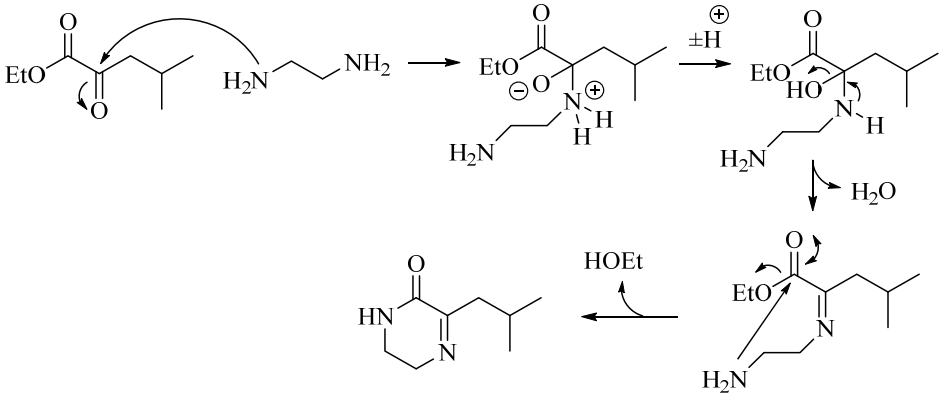


Scheme 15. Mechanism of the preparation of ethyl 4-methyl-2-oxopentanoate (**58**) from diethyl oxolate.

In the literature, dry THF and Et_2O are used solely or in a 1:1 mixture for equivalent transformations. The reaction temperature was manually controlled by slow addition of the Grignard as the reaction was noted to be exothermic. As an illustration, upon dropwise addition of isobutyl magnesium bromide, the temperature rises from -78 °C to above -70 °C in the first few seconds. After evaluating and comparing a series of experimental runs, reactions in which the dropwise addition temperature was maintained below -70 °C gave the highest yields of the desired 5,6-dihydropyraizne derivative **41**. To gain greater control over the addition rate a dropping funnel set-up was used (Figure 21) instead of the original cannula reagent transfer. The desired 4-methyl-2-oxopentanoate (**58**) was detected by GC-MS at R_t = 2.76 min [M] = 158.2 and carried directly into next step as it was noted to decompose upon storage.

Figure 21. The set up for ethyl 4-methyl-2-oxopentanoate (**58**) preparation under dry condition (N_2 balloons not shown).

2.2.2 Preparation of 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (41)



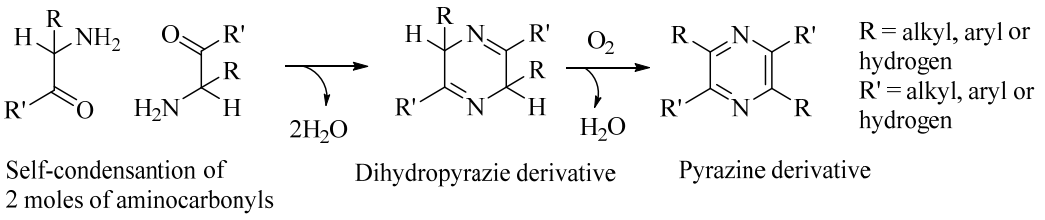
Scheme 16. The proposed mechanism of 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**41**) preparation.

Having successfully prepared the desired 4-methyl-2-oxopentanoate (**58**) we next turned our attention to its condensation (Scheme 16). We tried two different amine sources, namely, ethylenediamine and ethylenediamine dihydrochloride for the condensation procedure. The 1,2-diamine was added directly to the extracted organic layer of freshly prepared ethyl 4-methyl-2-oxopentanoate (**58**), as we observed it to decompose when stored overnight and this had a significant impact on the final isolated

yield of **41**. In reaction with the ethylenediamine dihydrochloride it was found the salt did not fully dissolve even when left to stir for a full 64 h at room temperature (the assumption had been it may slowly go into solution as it reacted). As such this diamine source was therefore change in preference to ethylenediamine (the liquid form of the reagent). Addition of this reagent immediately showed the formation of the desired product by GC-MS analysis. However, the best yield obtained for this reaction was only 46%.

2.2.3 Aromatisation of 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (41)

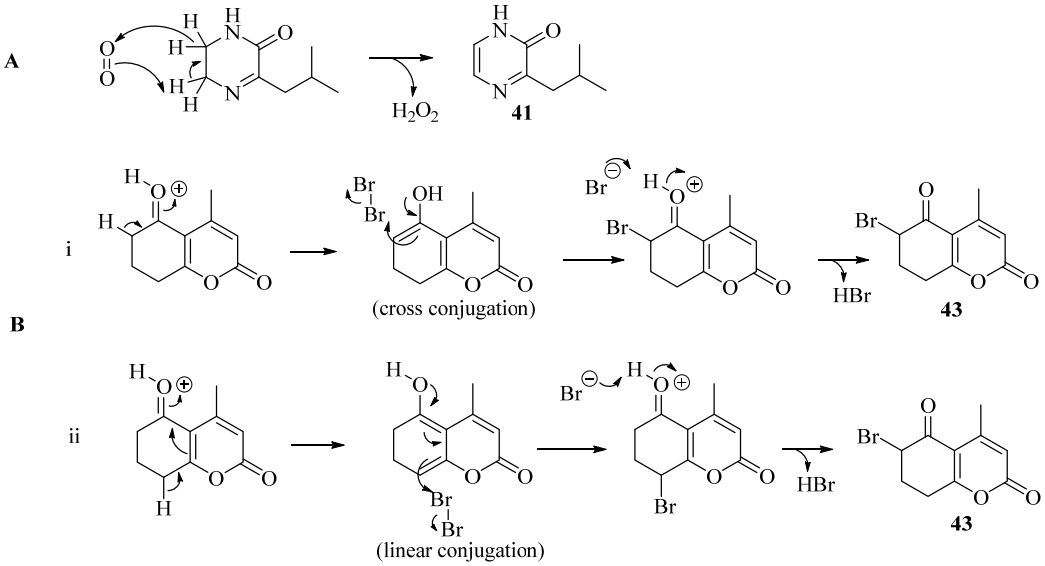
Initially the potential for autooxidation of 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**41**) was tested with a sample left in contact with air, indication in the literature was slow spontaneous oxidation could occur on such systems (Scheme 17).[12] However, the GC-MS analysis did not show the molecular ion peak of the desired 2-isobutyl-3-methxoypyrazine (**21**) even after 6 months at room temperature (February to August 2021 – testing facilitated by a COVID shutdown). An additional sample (dihydropyrazine) was dissolved in methanol and a catalytic amount of potassium *tert*-butoxide was added whilst the system was placed under an oxygen atmosphere (balloon). Again, no molecular ion peak of the desired pyrazine derivative was observed in this sample by GC-MS analysis (1-7 days). A range of further oxidants were also tested for this process, including ceric ammonium nitrate, 2,3-dichloro-5,6-dicyano-1,4-benzoquinon, trichloroisocyanuric acid, manganese dioxide, dimethyl sulfoxide/I_2, potassium peroxymonosulfate and sodium hydride/O_2. However, under none of these conditions was the desired pyrazine derivatives identified by ^{1}H-NMR, GC-MS or LC-MS analysis. The reason could be because of the activation energy required for this procedure is much higher than expected. Basic 3D conformational analysis did not seem to indicate any particular reason based upon shape or alignment of groups that would prevent the desired oxidation in this structure when several related systems had been oxidised successfully in the literature.



Scheme 17. Pyrazine preparation scheme from condensation of 2 moles of 2-aminocarbonyls.[12]

2.2.3.1 Aromatisation of 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (43) via halogenation synthesis

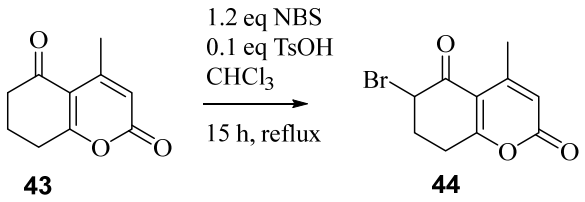
As the oxidation of the 5,6-dihydropyrazine derivative **41** was proving problematic (and synthesised material was running low), we elected to explore methods based upon a model substrate. We selected the compound 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**43**) as this was also only a single oxidation from being aromatic. The oxidations of both compounds therefore had very similar requirements. The hydrogen abstraction, as required for the 5,6-dihydropyrazine (Scheme 17A) also failed under similar conditions when using this new substrate. We therefore tested another route which overall eliminates the hydrogen of the model compound **43**. In principle, the bromination of compound **43** can occur at the *ortho* position via enol in the cross-conjugation form (Scheme 18B i) or at the *para* position via an enol in linear conjugation (Scheme 18B ii).



Scheme 18. **A**. An example of proposed oxidation mechanism of 5,6-dihydropyrazine (**41**). **B**.

Proposed oxidation mechanism of model compounds 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**43**).

The procedure followed was based on the work of Vyas *et al*. 2016, where the oxidation of tetralone was established.[67] By using 1.2 equivalents of *N*-bromosuccinimide and 0.1 equivalents of *p*-toluenesulfonic acid monohydrate, 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**) and 8-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**59**) was detected by LC-MS at retention times of 5.62 minutes and 5.77 minutes, whilst a peak at 5.06 minutes which had a molecular ion peak of the starting material was seen to slowly diminish (Scheme 19, Figure 22).



43 **44**

Scheme 19. The bromination of 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**43**) to 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**).

Additional optimisation was performed exploring the equivalents of *N*-bromosuccinimide (NBS) and solvent in order to drive the reaction to completion (Table 15). The best result was obtained using 1.4 equivalents of NBS with chloroform as the solvent which also gave a clean GC-MS profile upon scaling of the reaction (Figure 23).

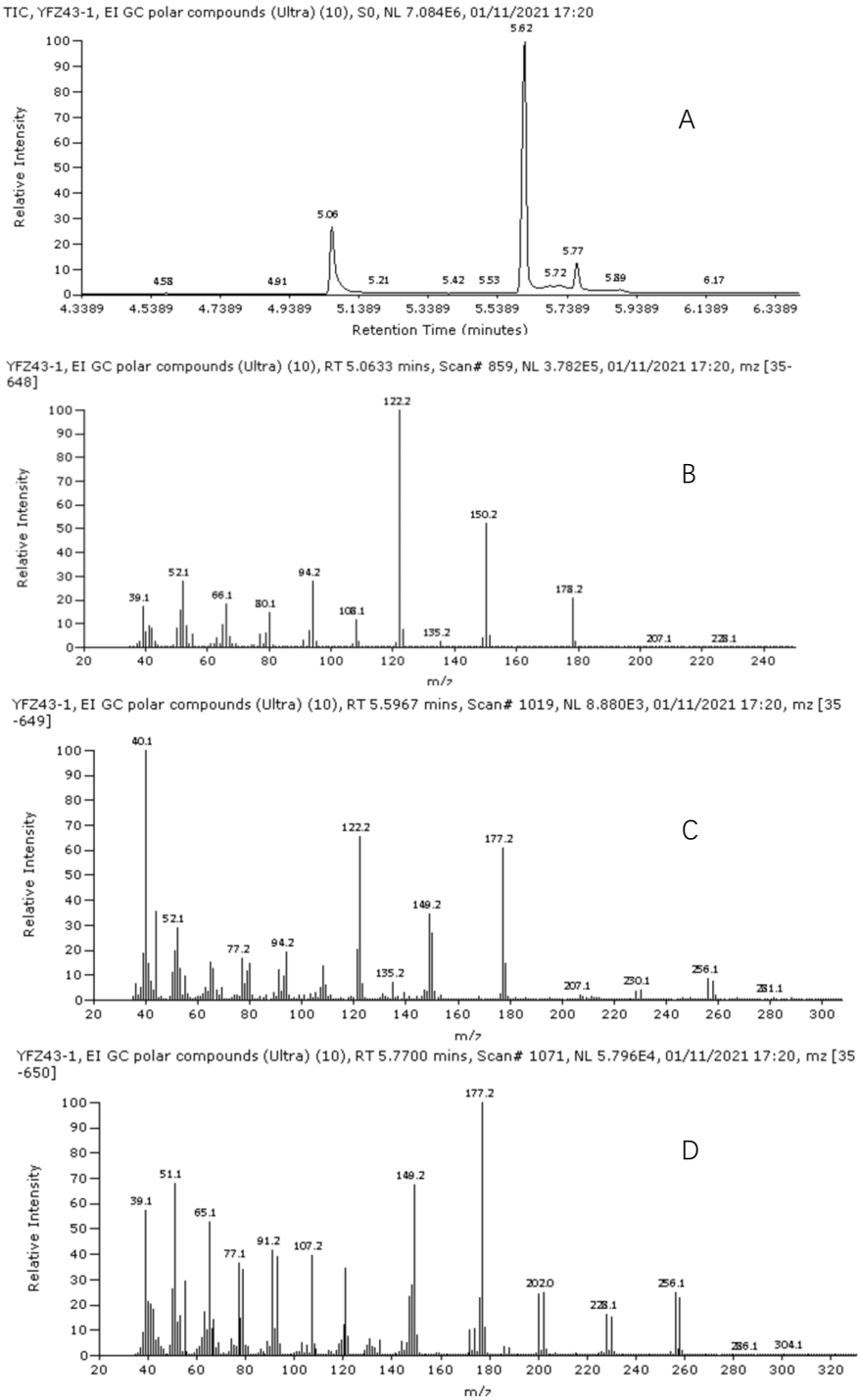


Figure 22. **A** GC-MS retention time of molecular ion peaks for Scheme 19. **B**. Indication of the molecular ion peak of the starting reagent 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**43**). **C** The major molecular ion peak of proposed 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(*6H*)-dione (**44**), m/z = 256.1, R_t = 5.62 min. **D** The minor molecular ion peak of proposed 8-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**64**), m/z = 256.1, R_t = 5.77 min.

Table 15. The reaction results of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**) preparation.

Entry	Scale/ mmol	Eq. of NBS	Solvent	Time	Relative intensity of starting material peak
1	10	1.2	CH_2Cl_2	2 overnights	20
2	10	1.3	CH_2Cl_2	2 overnights	5
3	10	1.4	CH_2Cl_2	2 overnights	2
4	50	1.4	$CHCl_3$	1 overnight	Close to 0
5	10	1.4	MeCN	1 overnight	70

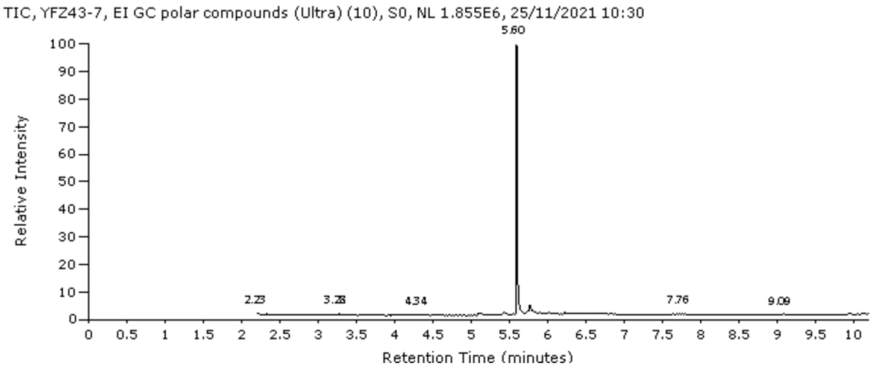
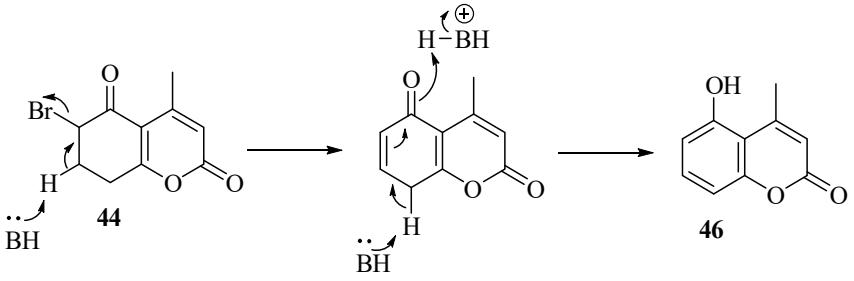


Figure 23. The GC-MS of the product with conditions in Table 15 Entry 4, the peak for starting reagent was diminish completely at 5.06 min.

The second step of the sequence requires elimination of the newly installed halogen. The base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was selected to perform the elimination of compound **44** because the bulky structure of the base in principle favours the E2 elimination whilst avoiding competing S_N2 mechanism due to steric hinderance effects. The proposed mechanism is shown below in Scheme 20.



Scheme 20. The proposed mechanism for elimination of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-

2,5(6*H*)-dione (**44**).

Initial analysis by GC-MS showed the existance of two closely eluting molecular ion peaks, indicative of the desired 5-hydroxy-4-methylcoumarin (**46**) and another bromo containing compound since an isotopic peak with 1:1 ratio was observed (Figure 24).

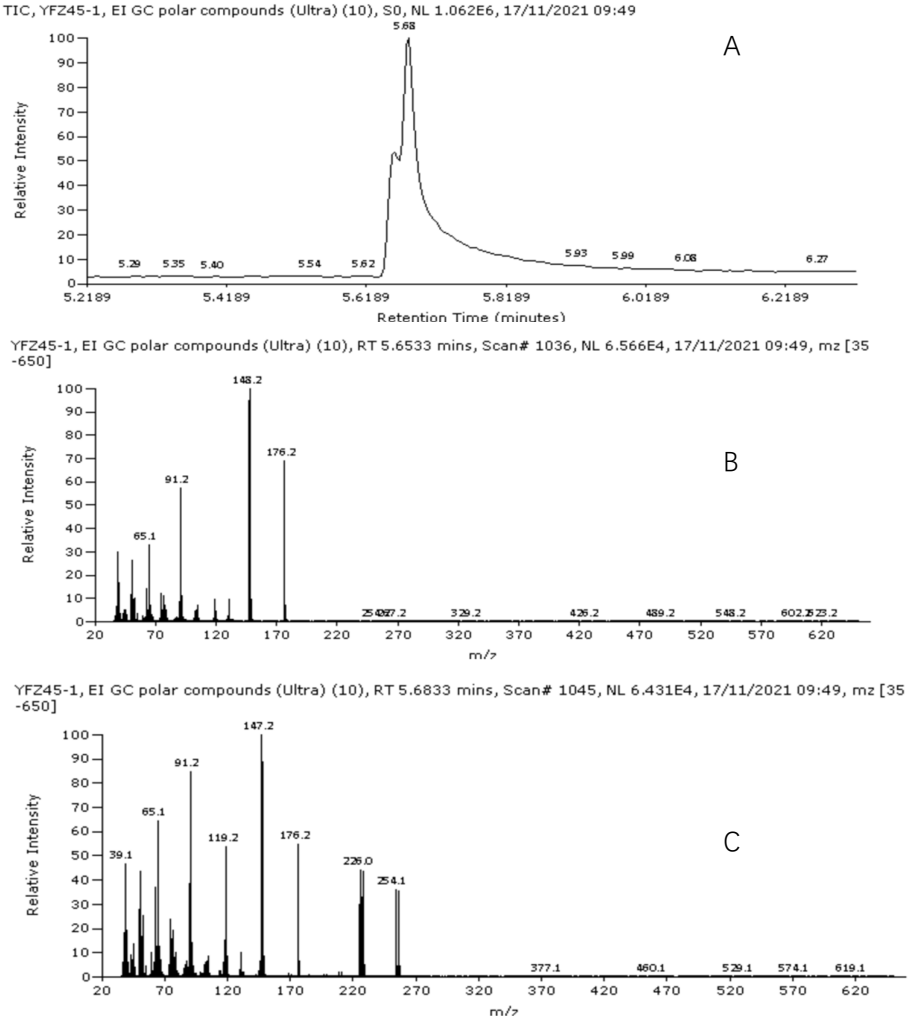
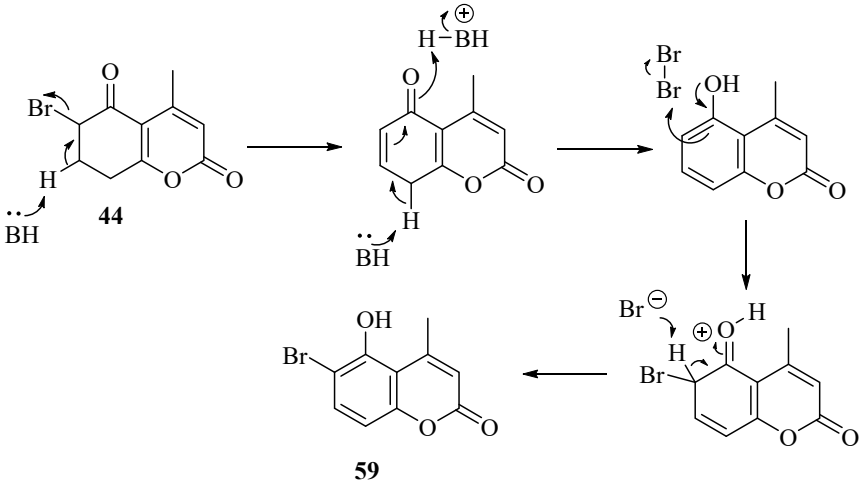


Figure 24. The GC-MS analysis of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**). **A** The detected peaks at retention time. **B** The molecular ion peak indicating 5-hydroxy-4-methylcoumarin (**46**) at m/z = 176.2, R_t = 5.65 min. **C** The molecular ion peak indicating 6-bromo-5-hydroxy-4-methylcoumarin (**44**) at m/z = 176.2, R_t = 5.65 min.

Considering the presence of bromide ions in the solution mixture, we propose that Br_2 could be being formed by oxidation of bromide ions by air, then following aromatisation (via elimination) a secondary bromination is thus possible (Scheme 21). The selectivity for the resulting mono-brominated product 6-bromo-5-hydroxy-4-methylcoumarin (**44**) over the potential secondary site leading to 8-bromo-5-hydroxy-

4-methylcoumarin (**59**) is due to lone pair repulsion on the adjacent oxygen atom at the position 9 (Figure 25) for this latter compound. Interestingly, when running the reaction under an inert atmosphere and sealing the condenser with septum and nitrogen balloon we still did not obtain pure 5-hydroxy-4-methylcoumarin (**46**) (Figure 26). Due to time constraints additional degassing and supplemental oxygen additional was not tested further, however, it would be useful to perform a series of calibration reactions to correlate this result.



Scheme 21. The mechanism of aromatisation of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**).

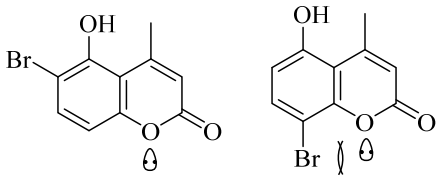


Figure 25. The steric hinderance illustration of 8-bromo-5-hydroxy-4-methylcoumarin (**59**).

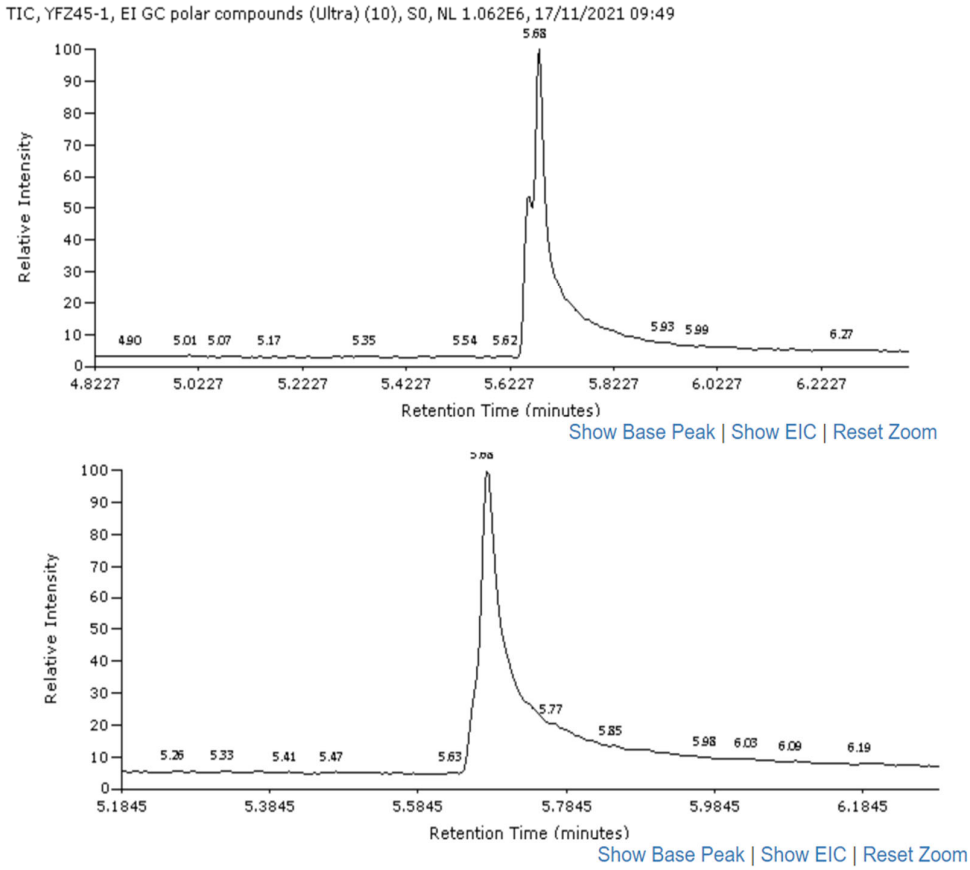
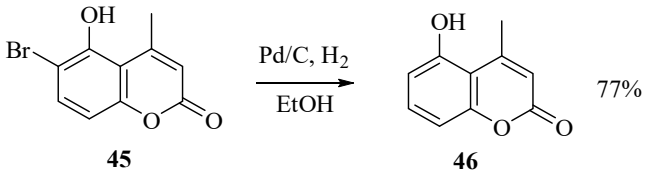


Figure 26. The comparison of 6-bromo-4-methyl-7,8-dihydro-2H-chromene-2,5(6H)-dione (**45**) ratio without and with degassing of oxygen in reaction system by GC-MS. For both cases, at R_t = 5.68 min, m/z = 254.1 and 256.1.

To confirm the identity of the brominated adduct a further dehalogenation step was performed to obtain pure 5-hydroxy-4-methylcoumarin (**46**) (Scheme 22).



45 **46**

Scheme 22. The dehalogenation reaction scheme of 6-bromo-5-hydroxy-4-methylcoumarin (**45**) to 5-hydroxy-4-methylcoumarin (**46**).

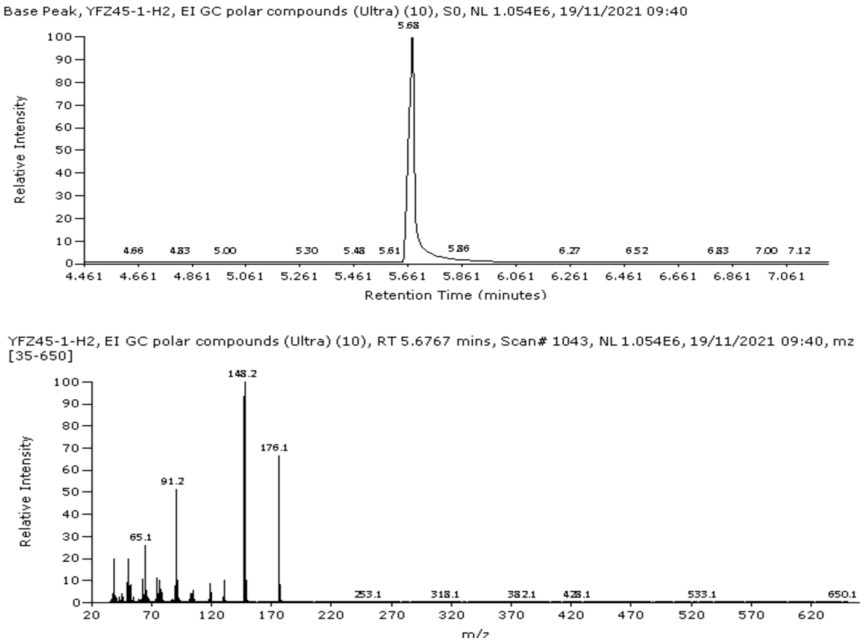
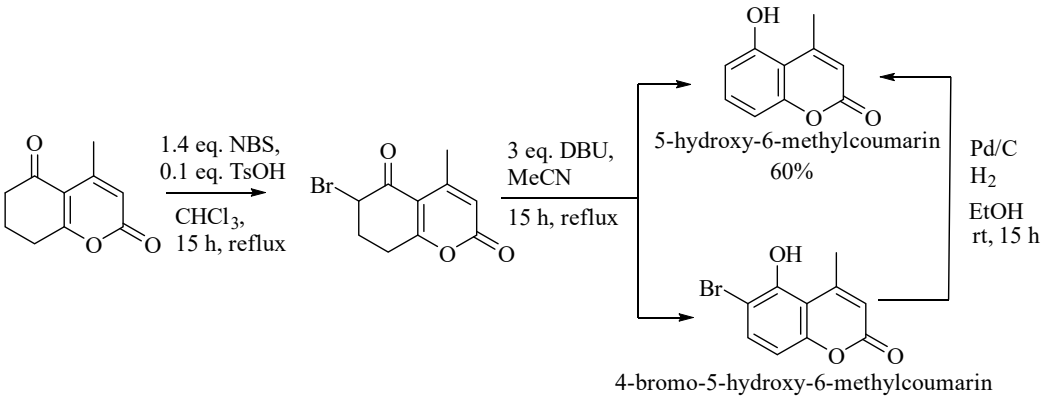


Figure 27. The GC-MS analysis of reaction in Scheme 22. A clean peak is proposed to be 5-hydroxy-4-methylcoumarin at R_t = 5.68 min, m/z = 176.1.

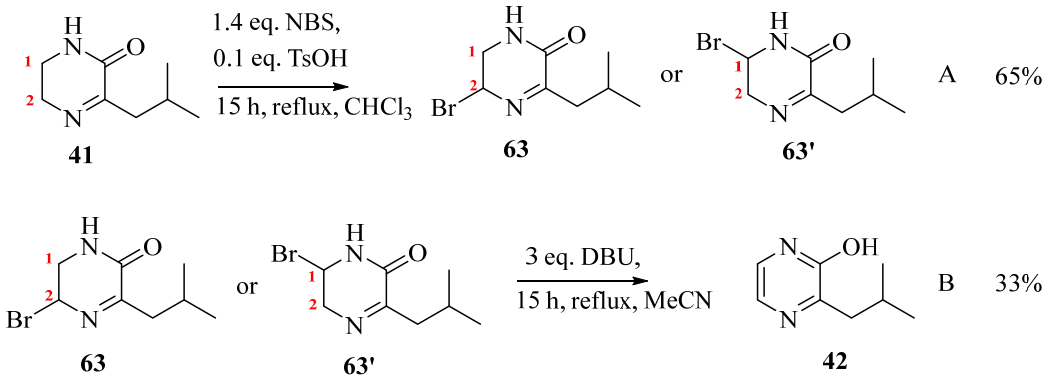
Thus, the overall reaction scheme for the preparation of 5-hydroxy-6-methylcoumarin (**46**) is shown in Scheme 23.



Scheme 23. The overall reaction scheme of aromatisation from 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**43**) to 5-hydroxy-6-methylcoumarin (**46**).

2.2.3.2 Aromatisation of 3-isobutyl-5,6-dihydropyrazine (41) via the halogenation protocol

The model synthetic procedure previously used for preparing 5-hydroxy-6-methylcoumarin (**46**) as explained in Scheme 23 was also successfully applied to the aromatisation of compound **63** or **63'** and afforded the desired **42** (Scheme 24).



Scheme 24. **A**. The bromination of 3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**41**) to 5-bromo-3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**63**) or 6-bromo-3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**63'**). **B**. 5-bromo-3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**63**) aromatisation of 6-bromo-3-isobutyl-5,6-dihydropyrazine-2(1*H*)-one (**63'**) to 2-isobutyl-3-hydroxypyrazine (**42**).

The compound **42** was confirmed by comparison to known data from section 2.1.3, detailed data analysis can be found in the Experimental section. The brominated intermediate (either **63** or **63'**) was characterised by GC-MS (Figure 28). Isotopic peaks at m/z = 232.0 and 234.0 (1:1) provided the evidence for bromination. The ^{1}H NMR of starting material and product of Scheme 24 showed the expected hydrogen integral change for substitution at carbon (Figure 29 and Figure 30). In other words, the bromide was substituted at either position 1 or 2 and thus afforded compound **63** or **63**'respecively. It is possible as illustrated by mechanisms disclosed in Scheme 25 for a bromide to be substituted on nitrogen to afford **63''** since this is potentially the most nucleophilic centre (Scheme 25B). This is however, contrary to the NMR evidence of the loss of a proton at either carbon 1 or 2 (Scheme 24). Although further bromination could afford **63'**, the tautomerisation product **63'''** would be more favoured by thermodynamics but lacks aromatic hydrogens in the NMR. Hence, compound **63'** was considered less likely to be the intermediate but no experimental evidence currently exists to confirm this hypothesis. Scheme 25A details the formation of compound **63** and this is consistent with the analysis data. Further analysis such as Heteronuclear

Multiple-Quantum Correlation (HMQC) using a ^{15}N correlation could potentially be used to analyse the position of substituted bromide, however, this was not performed due to time constraints on the project.

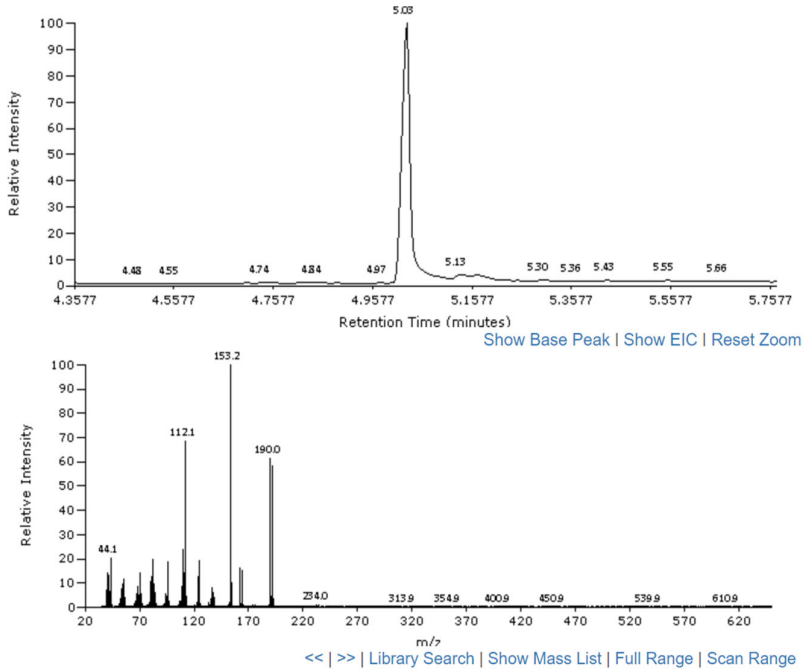


Figure 28. The GC-MS evidence of formation of **63** at R_t = 5.03 min where isotopic m/z = 232.0 and 234.0.

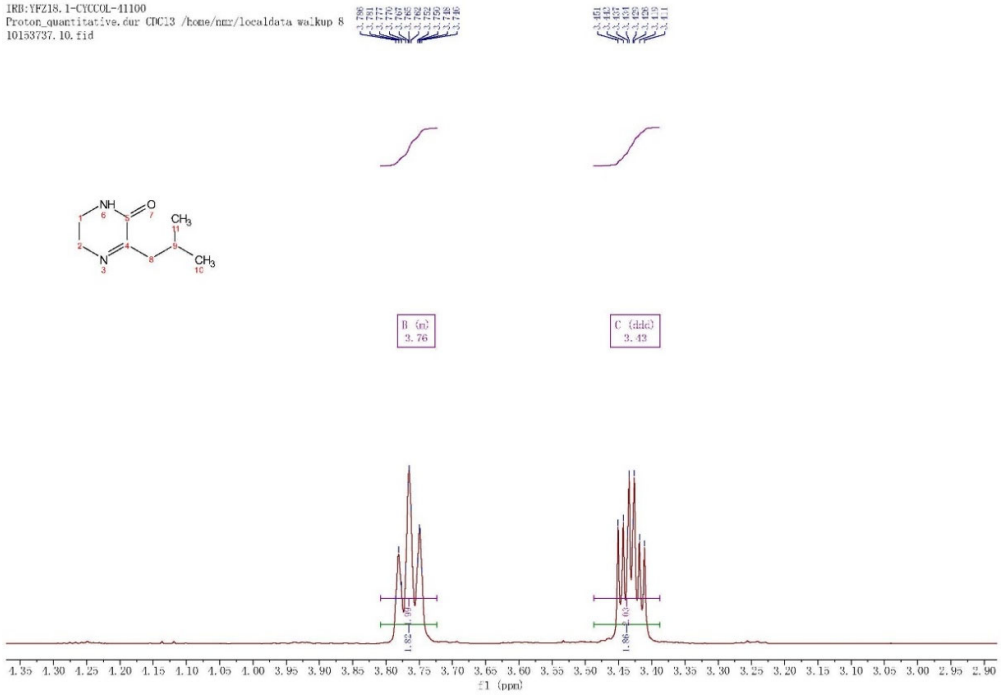


Figure 29. The ^1H NMR integral ratio or hydrogens at position 1 and 2 of **41** is approximately 1:1.

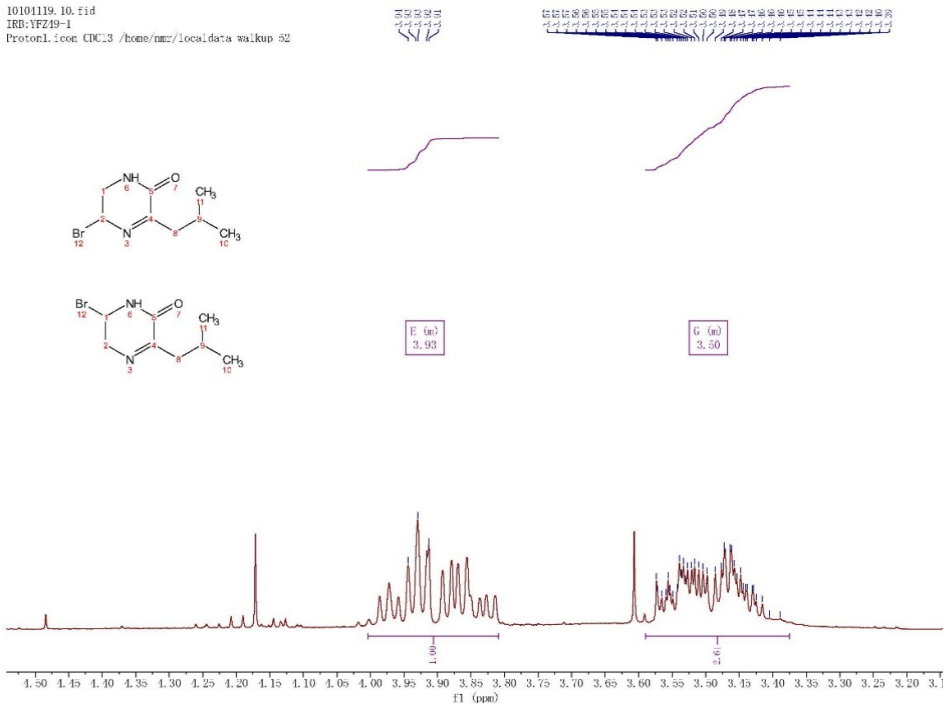
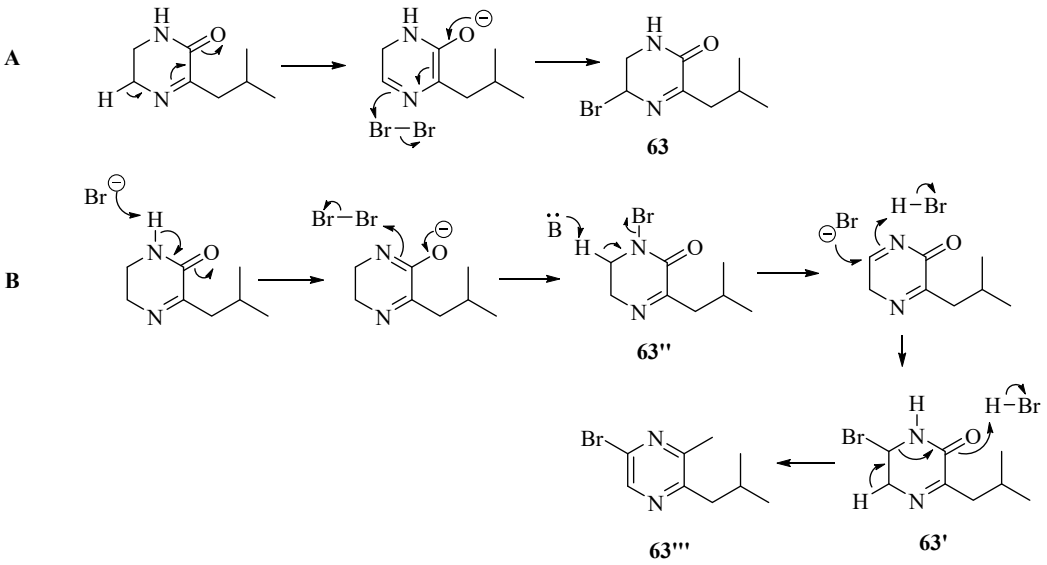
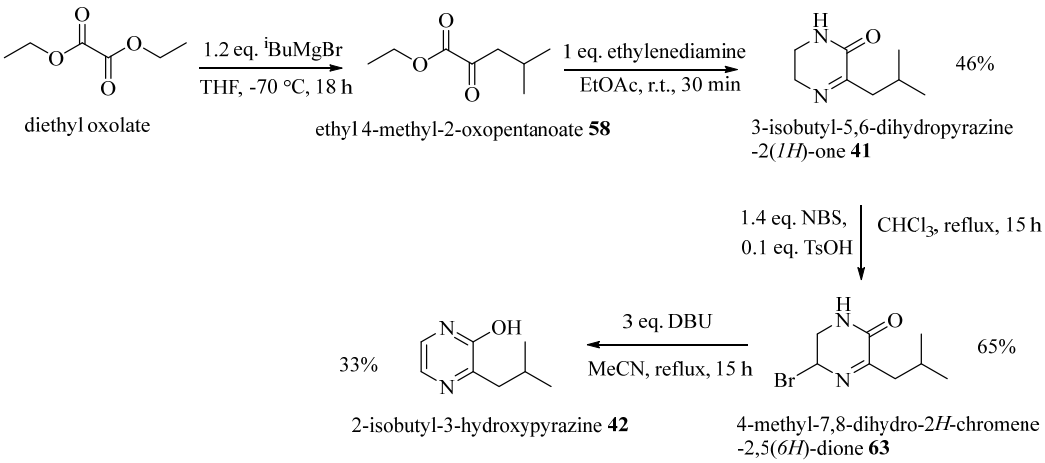


Figure 30. The ^{1}H NMR integral ratio or hydrogens at position 1 and 2 of **63 or 63'** is approximately 2:1 or 1:2.



Scheme 25. The proposed mechanism for the formation of **63**, **63'**, **63''** and **63'''**.

In summary, a novel preparation of 2-isobutyl-3-hydroxypyrazine (**42**) was established by condensation of a prepared diketone and commercially available diamine reagents followed by aromatisation via bromination of dihydropyrazine compound **41** as shown in Scheme 26.



Scheme 26. **A** The synthesis of 2-isobutyl-3-methoxypyrazine (**21**) from diethyl oxalate.

3 Conclusion and proposed future work

In general, the aim of improving upon the literature preparation of 2-isobutyl-3-methxoypyrazine (**21**) has been achieved including the establishment of a novel methylation protocol using a solid supported reagent. Overall, the yield of **21** has been increased from 19% as previously reported in the literature to 50% in this project (NOTE: The conversion of *L*-leucine to *L*-leucine methyl ester hydrochloride has not been recorded by Seifert *et al*., 1970[1]). The stepwise conversion of *L*-leucine to *L*-leucine methyl ester hydrochloride, *L*-leucinamide hydrochloride, to 2-isobutyl-3-hydroxypyrazine were improved based on reviewed literature employing synthesis conditions without recourse to hazardous gases or harsh reaction conditions but achieving good to excellent yields. A novel methylation of 2-isobutyl-3-methoxypyrazine to 2-isobutyl-3-methoxypyrazine was designed with Ambersep 900 OH form under room temperature condition. The simple work up procedure was completed by concentrating the solution *on vacuo*. The application of 2-*sec*-butyl-3-methoxypyrianze (**53**) and 2-isopropyl-3-methoxypyrazine (**56**) synthesis gave 54% and 26% yield respectively. The ester chain length of amino acid ester hydrochloride was found highly influential on *L*-leucinamide hydrochloride formation, methyl ester was favoured over ethyl ester.

4 Experimental Methods

General Information

Unless specified, reagents were obtained from commercial sources and used without further purification. Solvents were obtained from Fischer Scientific.

Melting point

Melting points were recorded on an Optimelt automated melting point system and are uncorrected. The heating ramp gradient was set at 1 °C min^{-1}.

Chromatography

Flash chromatography was performed using Merck Silica gel high-purity grade (9385), pore size 60 Å, 230-400 mesh particle size. Thin-Layer Chromatography was performed using Merck TLC silica gel 60 with glass support. IR spectra were recorded neat on a Perkin-Elmer Spectrum Two FT-IR spectrometer. The absorbency of the peaks was defined as: weak (w, < 40% of most the intense peak), medium (m, 40 - 75% of the most intense peak), strong (s, > 75% of the most intense peak) and broad (br).

NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III-HD-400 spectrometer with operating frequencies of 400 MHz for ^{1}H, 101 MHz for ^{13}C.

Proton chemical shift values are given in units δ relative to residual protic solvent. The multiplicity of the signal is indicated as: br – broad, s – singlet, d – doublet, t – triplet, q – quartet and m – multiplet, dd – doublet of doublets, dt – doublet of triplets, etc. Coupling constants (J) were measured to the nearest 0.1 Hz. Carbon chemical shift data are given in units δ relative to residual protic solvent.

Mass spectroscopy

Liquid chromatography-mass spectrometry (LCMS) was performed on an TQD mass spectrometer and an Acquity UPLC (Waters Ltd, UK). GCMS experiments were carried out on a Shimadzu QP2010-Ultra.

X-ray crystal analysis

X-Ray crystal structure determination was performed by Dr Dmitry Yufit at the

Department of Chemistry, University of Durham on a Bruker D8 Venture diffractometer with PHOTON 100 CMOS area detector, using Mo-Kα radiation from Incoatec IμS microsources with focusing mirrors. The crystals were cooled using a Cryostream 700 (Oxford Cryosystems, (Oxford, Oxfordshire, UK) open-flow N_2 gas cryostat. The structures were solved by dual-space intrinsic phasing (SHELXT program)[68] and refined by full-matrix least squares using SHELXL[69] software on Olex2 platform.[70]

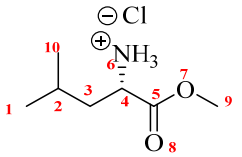
Flow reactors

Flow reactions were performed using a commercially available system from Vapourtec Ltd comprising of six peristaltic pumps.

4.1 General procedure A: Synthesis of amino acid methyl ester hydrochloride

To a solution of amino acid (10 mmol) in methanol (10 mL) was added chlorotrimethylsilane (20 mmol) and the mixture stirred at room temperature for 24 h. After the completion of reaction as monitored by TLC (EtOAc), the solvent was removed by evaporation under a reduced pressure to obtain the amino acid methyl ester hydrochloride (**49**).

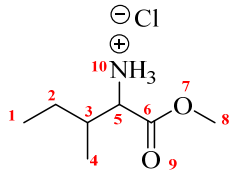
***L*-leucine methyl ester hydrochloride (49)**



Chemical Formula: $C_7H_{16}ClNO_2$
Exact Mass: 181.0870

Pale yellow powder, 1.68 g (93%), mp. 125-127.5 °C, (Lit. 127 – 128 °C[71]). ν_{max}/ cm^{-1} 2956 (m, NH), 1736 (s, CO); δ_H (400 MHz, D_2O) 4.03 (t, 1H, H4), 3.72 (s, 3H, H9), 1.74 (m, 1H, H2), 1.62 (m, 2H, H3), 0.83 (6H, m, H1+H10); δ_C (101 MHz, D_2O) 171.27 (C5), 53.42 (C9), 51.35 (C4), 38.71 (C3), 23.84 (C2), 21.40 (C1 or C10) 20.89 (C1 or C10); LC-MS R_t = 0.26 min, 146.8 $[M]^+$.

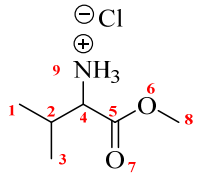
***L*-isoleucine methyl ester hydrochloride (51)**



Chemical Formula: $C_7H_{16}ClNO_2$
Exact Mass: 181.0870

Pale orange solid, 1.75 g (97%), m.p. 85-88 °C. ν_{max}/ cm^{-1} 2880 (br, NH), 1736 (s, CO); δ_H (400 MHz, D_2O) 4.00 (d, J = 4.0 Hz, 1H, H4), 3.73 (s, 3H, H8), 1.96 (m, 1H, H3), 1.35 (m, J = 14.7, 7.4, 5.5 Hz, 1H, H2), 1.21 (m, 1H, H2), 0.89 (d, J = 7.0 Hz, 3H, H4), 0.82 (t, J = 7.4 Hz, 3H, H1); δ_C (101 MHz, D_2O) 170.25 (C6), 57.19 (C8), 53.23 (C5), 35.92 (C3), 24.72 (C2), 13.99 (C4), 10.74 (C1); LC-MS R_t = 0.24 min, m/z = 146.4 $[M]^+$.

***L*-valine methyl ester hydrochloride (54)**



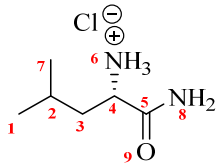
Chemical Formula: $C_6H_{14}ClNO_2$
Exact Mass: 167.0713

A total of 8.29 g (99%) was prepared from 50 mmol of *L*-valine methyl ester hydrochloride using General procedure A as white powder, mp. 161-164 °C. ν_{max}/ cm^{-1} 2834 (br, NH), 1736 (s, CO); δ_H (400 MHz, D_2O) 3.95 (d, J = 4.7 Hz, 1H, H4), 3.76 (s, 3H, H8), 2.26 (m, J = 7.1, 4.7 Hz, 1H, H2), 0.94 (dd, J = 7.0, 5.9 Hz, 6H, H1+H3); δ_C (101 MHz, D_2O) 170.34 (C5), 58.34 (C8), 53.35 (C4), 29.25 (C2), 17.25 (C1 or C3), 16.94 (C1 or C3); LC-MS R_t = 0.24 min, m/z = 132.3 $[M]^+$.

4.2 General procedure B: Synthesis of amino acid amide hydrochloride

To the amino acid methyl ester hydrochloride (10.0 g, 55 mmol) was added 35% aqueous ammonia (50 mL). The reaction mixture was stirred at 55 °C for 6 h under inert atmosphere. The solution was allowed to cool and crystalise, the solid impurities were removed by filtration. The filtrate was evaporated under reduced pressure to afford the aminoacid amide.

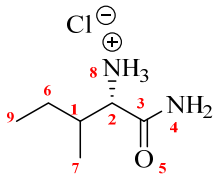
L-Leucinamide hydrochloride (50)



Chemical Formula: $C_6H_{15}ClN_2O$
Exact Mass: 166.09

Pale pink solid, 8.1 g (89%), mp. 231-235 °C; ν_{max}/ cm^{-1} 3174-2871 (br, NH), 1673 (CO) cm^{-1}; δ_H (400 MHz, d_6-DMSO), 8.09 (s, 1H, H8), 7.51 (s, 1H, H8), 3.68 (t, 1H, H4), 1.68 (m, 1H, H2), 1.57 (m, 2H, H3), 0.89 (m, 6H, H1+H7); δ_C (101 MHz, d_6-DMSO) 171.30 (C5), 51.29 (C4), 31.19 (C2), 24.15 (C3), 22.95 (C1 or C9), 22.69 (C1 or C7). LC-MS R_t = 0.24 min, m/z = 131.4 $[M]^+$.

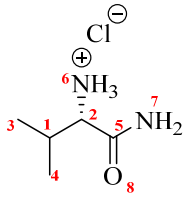
L-isoleucinamide hydrochloride (52)



Chemical Formula: $C_6H_{15}ClN_2O$
Exact Mass: 166.0873

Pale orange solid, 6.65 g, (81 %), 221-225 °C. ν_{max}/ cm^{-1} 2962 (NH), 1697 (CO), 1606 (NH); δ_H (400 MHz, d_6-DMSO) 7.93 (s, 1H, H4), 7.53 (s, 1H, H4), 3.58 (d, J = 5.2 Hz, 1H, H2), 1.84 (s, 1H, H1), 1.51 (s, 1H, H6), 1.12 (m, J = 15.4, 7.8 Hz, 1H, H6), 0.89 (m, 6H, H9+H7); `δ_C (101 MHz, d_6-DMSO) 170.21 (C3), 57.03 (C2), 36.37 (C1), 24.59 (C6), 15.12 (C9 or C7), 11.81 (C9 or C7); LC-MS R_t = 0.24 min, m/z = 131.6 $[M]^+$.

L-Valinamide hydrochloride (55)



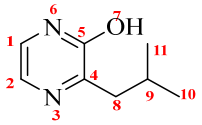
Chemical Formula: $C_5H_{13}ClN_2O$
Exact Mass: 152.0716

A 30 mmol fraction of *L*-valine methyl ester hydrochloride was reacted with 25 mL of 35% aqueous ammonia using General procedure B to afford *L*-valinamide hydrochloride in 3.66 g (82%) as white powder, mp. 241-245 °C; ν_{max}/ cm^{-1} 2971 (NH), 1635 (CO), 1320 (CN); δ_H (400 MHz, d_6-DMSO) 7.97 (s, 1H, H4), 7.54 (s, 1H, H4), 3.55 (d, J = 5.4 Hz, 1H, H2), 2.11 (m, J = 13.4, 7.0 Hz, 1H, H1), 0.94 (t, J = 6.8 Hz, 6H, H3+H4); δ_C (101 MHz, d_6-DMSO) 170.20 (C5), 57.79 (C2), 29.89 (1), 18.85 (C3), 18.19 (C4); LC-MS R_t = 0.27 min, m/z 117.2 $[M]^+$.

4.3 General procedure C: Synthesis of 2-alkyl-3-hydroxypyrazine

A solution of amino acid amide hydrochloride (10 mmol) in methanol (20 mL), was cooled to -35 °C by cooling bath made from 35% water in methanol. To the reaction mixture was added glyoxal (5 mL, 40% aqueous solution of glyoxal) with rapid stirring. Then sodium hydroxide aqueous solution (12 M) was added dropwise until the pH of solution was 10 ~12. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Neutralisation was performed by adding hydrochloric acid (12 M). Methanol was removed under reduced pressure and water (20 mL) was added. The organic phase was extracted with dichloromethane (3 × 50 mL), dried over Na_2SO_4, filtered and evaporated *in vacuo* to obtain the desired 3-alkyl-2-hydroxypyrazine.

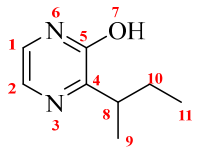
2-isobutyl-3-hydroxypyrazine (42)



Chemical Formula: $C_8H_{12}N_2O$
Exact Mass: 152.0950

Brown solid, 1.1 g, (67%), mp. 86 – 88 (Lit 90-92.5 [1]). δ_H (400 Hz, $CDCl_3$) 7.45 (d, J = 4.1 Hz, 1H, H1), 7.17 (d, J = 4.1 Hz, 1H, H2), 2.71 (d, J = 7.2 Hz, 2H, H8), 2.27 (m, 1H, H9), 0.99 (d, J = 6.7 Hz, 6H, H10+H11); δ_C (101 MHz, $CDCl_3$) 160.65 (C1 or C2), 157.97 (C1 or C2), 124.07 (C4 or C5), 123.64 (C4 or C5), 41.83 (C8), 26.62 (C9), 22.63 (C10+C11); GC-MS R_t = 4.32 min, m/z 152.2 [M].

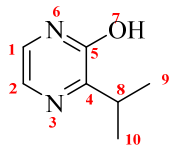
3-*sec*-butyl-2-hydroxypyrazine (53)



Chemical Formula: $C_8H_{12}N_2O$
Exact Mass: 152.0950

1.32 g (87 %). ν_{max}/ cm^{-1} 2965 (OH); δ_H (400 MHz, $CDCl_3$) 7.46 (d, *J* = 4.1 Hz, 1H, H1), 7.15 (d, *J* = 4.1 Hz, 1H, H2), 3.30 (m, 1H, H8), 1.84 (m, 1H, H8), 1.56 (m, 1H, H10), 1.28 (m, 1H, H10), 1.23 (d, *J* = 6.9 Hz, 3H, H9), 0.92 (t, *J* = 7.4 Hz, 3H, H11); δ_C (101 MHz, $CDCl_3$) 164.81 (C1), 157.66 (C2), 124.34 (C4), 123.22 (C5), 36.63 (C8), 27.46 (C10), 17.79 (C9), 11.93 (C11); GC-MS R_t = 4.15 min, m/z = 152.2 [M].

3-isopropyl -2-hydroxypyrazine (56)



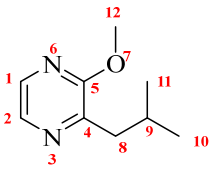
Chemical Formula: $C_7H_{10}N_2O$
Exact Mass: 138.0793

Isolated as a cream yellow solid in 0.53 g (39%), 72.5-75 °C. ν_{max}/ cm^{-1} 3074 (aromatic CH), 2974 (aromatic CH), 1636 (CO), 1586 (CC); δ_H (400 MHz, $CDCl_3$) 13.25 (s, 1H, H7), 7.45 (m, 1H, H1), 7.17 (m, 1H, H1), 3.46 (1H, H8), 1.27 (dd, *J* = 6.9, 1.4 Hz, 6H, H9+H10).; δ_C (101 MHz, $CDCl_3$) 165.10 (C1), 157.65 (C2), 124.35 (C4), 123.41 (C5), 30.18 (C8), 20.05 (C9+C10); GC-MS R_t = 3.76 min m/z 138.1 [M].

4.4 General procedure D: Synthesis of 3-alkyl-2-methoxypyrazine

The 3-isobutyl-2-methoxypyrazine (**21**) (0.50 g, 3.29 mmol) was dissolved in THF (20 mL) before addition of Ambersep 900 hydroxide form (7.00 g). The mixture was stirred for 10 min until the starting material was consumed as shown by TLC. Then dimethyl sulfate (0.83 g, 6.58 mmol) was added and the solution stirred for a further 2 h. The mixture was then filtered and the resin washed with THF. The product 3-alkyl-2-methoxypyrazine was obtained after concentrated the solution *on vacuo*.

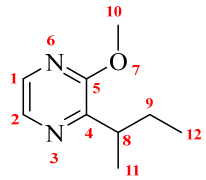
3-isobutyl-2-methoxypyrazine (**21**)



Chemical Formula: $C_9H_{14}N_2O$
Exact Mass: 166.1106

By using 1.00 g (6.5 mmol) of 3-isobutyl-2-hydroxypyrazine, 0.83 g (91%) of 3-isobutyl-2-methoxypyrazine was obtained as brown oil. ν_{max}/ cm^{-1} 2956 (CH), 1639 (C=N), 1595 (C=N); δ_H (400 Hz, $CDCl_3$) 7.21 (d, J = 4.4 Hz, 1H, H1), 7.01 (d, J = 4.4 Hz, 1H, H2), 3.50 (s, 3H, H12), 2.67 (d, J = 7.1 Hz, 2H, H8), 2.17 (m, 1H, H9) 0.91 (m, 6H, H10+H11); δ_C (101 MHz, $CDCl_3$) 160.34 (C1 or C2), 156.66 (C1 or C2), 127.72 (C3 or C4), 122.38 (C3 or C4), 42.22 (C8), 37.17 (C12), 26.53 (C9), 22.62 (C10+C11); GC-MS R_t = 4.22 min, 152.2 [M].

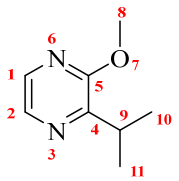
3-*sec*-butyl-2-methoxypyrazine (22)



Chemical Formula: $C_9H_{14}N_2O$
Exact Mass: 166.1106

By using 3-*sec*-butyl-2-hydroxypyrazine (0.34 g, 2 mmol), 0.29 g (77%) of 3-*sec*-butyl-2-methoxypyrazine was obtained as red-brown oil. ν_{max}/ cm^{-1} 2965 (aromatic CH), 1639 (C=N), 1589 (C=N); δ_H (400 MHz, $CDCl_3$) 7.26 (d, J = 4.4 Hz, 1H, H1), 7.00 (d, J = 4.4 Hz, 1H, H2), 3.53 (s, 3H, H10), 3.34 (m, J = 6.9 Hz, 1H, H8), 1.80 (m, 1H, H9), 1.52 (m, 1H, H9), 1.21 (m, 3H, H11), 0.90 (t, J = 7.4 Hz, 3H, H12); δ_C (101 MHz, $CDCl_3$) 164.54 (C1 or C2), 156.32 (C1 or C2), 127.36 (C4 or C5), 122.48 (C4 or C5), 37.25 (C8), 36.94 (C10), 27.51 (C9), 17.75 (C11), 11.95 (C12); GC-MS R_t = 4.12 min, m/z 166.1 [M].

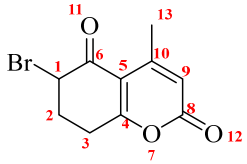
3-isopropyl-2-methoxypyrazine (20)



Chemical Formula: $C_8H_{12}N_2O$
Exact Mass: 152.0950

Brown yellow solid, 0.44 g (81%). ν_{max}/ cm^{-1} 3073 (aromatic CH), 2973 (aromatic CH), 1637 (C=N), 1590 (C=C); δ_H (400 MHz, $CDCl_3$) 7.22 (d, *J* = 4.3 Hz, 1H, H1), 7.00 (d, *J* = 4.3 Hz, 1H, H2), 3.50 (s, 3H, H8), 3.46 (m, 1H, H9), 1.20 (d, *J* = 6.9 Hz, 6H, H10+H11); δ_C (101 MHz, $CDCl_3$) 127.51 (C1), 122.43 (C2), 37.21 (C4), 30.44 (C5), 20.07 (C10+C11); LC-MS R_t = 0.26 min, m/z 153.6 [M].

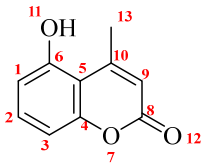
4.5 Synthesis of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (44)



Chemical Formula: $C_{10}H_9BrO_3$
Exact Mass: 255.9735

A solution of 4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (8.90 g, 50 mmol) in $CHCl_3$ (75 mL) was added dropwise to a solution of *N*-bromosuccinimide (12.45 g, 70 mmol) and *p*-toluenesulfonic acid monohydrate (0.95 g, 5.0 mmol) in $CHCl_3$ (50 mL) at room temperature. The reaction was then brought to reflux for 15 h. After cooling, addition of H_2O (100 mL), the organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 75 mL). The combined layers were washed with saturated aqueous Na_2CO_3 (75 mL) and brine (100 mL), dried over Na_2SO_4 and concentrated on vacuo to afford 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**44**) in more than 95% purity by GC-MS analysis. δ_H (400 MHz, DMSO) 6.20 (d, *J* = 1.4 Hz, 1H, H9), 3.59 (s, 3H, H13), 2.58 (m, 1H, H1), 2.50 (m, 2H, H2), 2.39 (m, 2H, H3); δ_C (101 MHz, *d*$_6$-DMSO) 188.98 (C8), 174.98 (C5), 158.95 (C4), 156.30 (C6), 113.05 (C9), 51.79 (C1), 28.50 (C3), 26.71 (C2), 22.39 (C13); GC-MS R_t = 5.60 min, m/z 256.1, 258.1 [M].

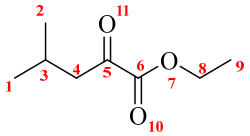
4.6 Synthesis of 5-hydroxy-4-methylcoumarin (46)



Chemical Formula: $C_{10}H_8O_3$
Exact Mass: 176.0473

To a solution of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**46**) in acetonitrile (20 mL) under an inert atmosphere was added 1,8-diazabicyclo[5.4.0]undec-7-ene (4.57 g, 30 mmol). The mixture was refluxed overnight (16 h) and cooled to room temperature before removing the solvent under reduced pressure. Water (200 mL) and 2% HCl (200 mL) was added to the crude mixture which was extracted by Et_2O (2 × 200 mL). The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford the intermediate. The intermediate was dissolved in EtOH (20 mL) and 10% palladium on activated charcoal (one 2 mL spoon spatula) under a hydrogen atmosphere for 4 h. The desired product was obtained by concentration of the filtrate *in vacuo*, 1.05 g (60% for the 2 steps), mp. ν_{max}/ cm^{-1} 3152 (OH), 1671 (C=O), 1606 (C=C); δ_H (400 MHz, d_6-DMSO) 10.68 (s, 1H, H11), 7.36 (t, J = 8.2 Hz, 1H, H2), 6.78 (dd, J = 8.2, 5.7 Hz, 2H, H1+H3), 6.14 (m, 1H, H9), 2.57 (s, 3H, H13); δ_C (101 MHz, d_6-DMSO) 160.14 (C8), 157.29 (C5), 155.31 (C4), 155.10 (C6), 132.56 (C2), 113.41 (C9), 111.69 (C1 or C3), 107.68 (C1 or C3), 24.13 (C13); GC-MS R_t = 5.67 min, m/z 176.1 [M].

4.7 Synthesis of Ethyl 4-methyl-2-oxovalerate (58)

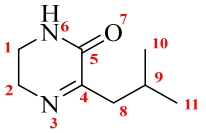


Chemical Formula: $C_8H_{14}O_3$
Exact Mass: 158.0943

Diethyl oxalate (24.11 g, 165 mmol) was dissolved in dry THF (180 mL). To the solution was added dropwise ^{i}BuMgBr (100 mL, 2.0 M in THF, 200 mmol, 1.2 eq.) at

-75 to -70 °C. The mixture was allowed to stir overnight (18 h). The mixture was warmed to room temperature and analysed by a mini work sample to check the formation of ethyl 4-methyl-2-oxovalerate (**58**). The mixture was quenched with saturated ammonium chloride (100 mL) and extracted with ethyl acetate (2 × 300 mL). The combined organic layers were dried over Na_2SO_4 and filtered for next procedure. δ_H (400 MHz, $CDCl_3$) 4.22 (m, 2H, H8), 3.47 (m, 1H, H3), 1.36 (m, 2H), 1.20 (t, J = 7.0 Hz, 3H, H9), 0.97 (m, 6H, H1+H2); δ_C (101 MHz, $CDCl_3$) 162.06 (C5), 65.83 (C6), 47.47 (C3), 24.25 (C4), 22.46 (C8), 17.45 (C9), 15.25 (C1 or C2), 13.88 (C1 or C2); GC-MS R_t = 2.79 min, m/z 158.2 [M].

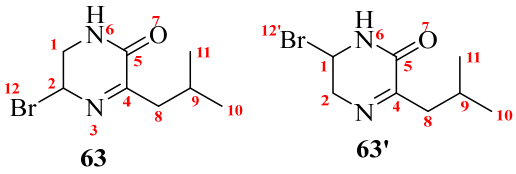
4.8 Synthesis of 2-hydroxy-3-isobutyl-4,5-dihydropyrazine (41)



Chemical Formula: $C_8H_{14}N_2O$
Exact Mass: 154.1106

One equivalent of ethylene diamine (9.92 g, 165 mmol) was added to a solution mixture containing ethyl 4-methyl-2-oxovalerate (**58**) and allowed to stir for 30 min. The mixture was concentrated *in vacuo* and purified by flush chromatography using hexane and ethyl acetate (from 1:1 to pure ethyl acetate) to obtain a yellow solid of 2-hydroxy-3-isobutyl-4,5-dihydropyrazine (**41**) (12.02 g, 46%). ν_{max}/ cm^{-1} 3193 (m, NH), 2954 (CH), 2870 (CH), 1679 (C=N), 1627 (C=O); δ_H (400 MHz, $CDCl_3$) 7.26 (s, 1H), 3.81-3.72 (m, 2H), 3.43 (m, 2H), 2.48 (m, 2H), 2.07 (m, 1H), 0.95 (d, 6H); δ_C (101 MHz, $CDCl_3$) 166.06 (C5), 158.17 (C4), 47.62 (C2), 42.40 (C1), 39.02 (C9), 26.22 (C8), 22.52 (C10+C11); LC-MS R_t = 1.06 min, m/z 173.6 $[M]^+$.

4.9 Synthesis of 5-bromo-3-isobutyl-5,6-dihydropyrazine-2(*1H*)-one (63) and/ or 6-bromo-3-isobutyl-5,6-dihydropyrazine-2(*1H*)-one (63')

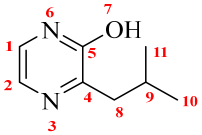


63 **63'**

Chemical Formula: $C_8H_{13}BrN_2O$
Exact Mass: 232.0211

A solution of 2-hydroxy-3-isobutyl-4,5-dihydropyrazine (**41**) (1.54 g, 10 mmol) in $CHCl_3$ (15 mL) was added dropwise to a solution of *N*-bromosuccinimide (2.14 g, 14 mmol) and *p*-toluenesulfonic acid monohydrate (0.19 g, 1 mmol) in $CHCl_3$ (10 mL) at room temperature. The reaction was then brought to reflux for 15 h. After cooling, the addition of H_2O (20 mL), the organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 15 mL). The combined organic layers were washed with saturated aqueous Na_2CO_3 (15 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford 4-methyl-7,8-dihydro-2H-chromene-2,5(6H)-dione (**63**). The mass is greater than 0.0207 g counting loss during transfer. δ_H (400 MHz, $CDCl_3$) 5.01 (d, J = 8.6 Hz, 1H), 3.93 (m, 1H, H2), 3.50 (m, 2H, H1), 2.46 (dp, J = 8.6, 6.6 Hz, 1H, H9), 1.27 (m, 2H, H8), 1.18 (d, J = 6.6 Hz, 3H, H10 or H11), 1.03 (d, J = 6.6 Hz, 3H, H10 or H11); δ_C (101 MHz, $CDCl_3$) 163.51 (C5), 156.59 (C4), 77.26 (C2), 55.90 (C1), 47.91 (C9), 38.67 (C8), 20.98 (C10 or C11), 20.55 (C10 or C11); GC-MS R_t = 5.02 m/z 232.0, 234.0 [M].

4.10 Synthesis of 3-isobutyl-2-hydroxypyrazine (42)



Chemical Formula: $C_8H_{12}N_2O$
Exact Mass: 152.0950

The 1,8-diazabicyclo[5.4.0]undec-7-ene (2.29 g, 15 mmol) was added to a solution of 6-bromo-4-methyl-7,8-dihydro-2*H*-chromene-2,5(6*H*)-dione (**63**) in acetonitrile (15 mL) under inert nitrogen atmosphere. The mixture was refluxed overnight (16 h) and cooled to room temperature before the solvent was removed under reduced pressure. The residues were extracted with water (100 mL), 2% HCl (110 mL) and Et_2O (2 × 100

mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to afford the 3-isobutyl-2-hydroxypyrazine (**42**). δ_H (400 MHz, $CDCl_3$) 7.44 (d, J = 4.1 Hz, 1H, H2), 7.16 (d, J = 4.1 Hz, 1H, H1), 2.71 (d, J = 7.1 Hz, 2H H8), 2.24 (m, 1H, H9), 0.98 (m, 6H, H10+H11); δ_C (101 MHz, $CDCl_3$) 160.76 (C1 or C2), 157.98 (C1 or C2), 124.32 (C4 or C5), 123.48 (C4 or C5), 41.86 (C9), 26.41 (C8), 22.53 (C10+C11); GC-MS R_t = 4.25 min, m/z 152.2 [M].

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